The Synthesis of Biologically Important Phase II Metabolites; 1-β-O-Acyl Glucuronides and 1-β-N^+-Glucuronides

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy.

by

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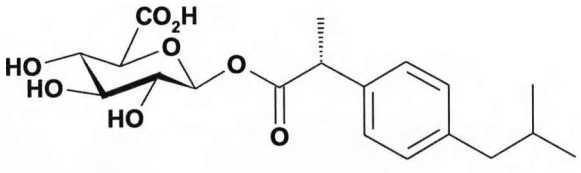
April 2009

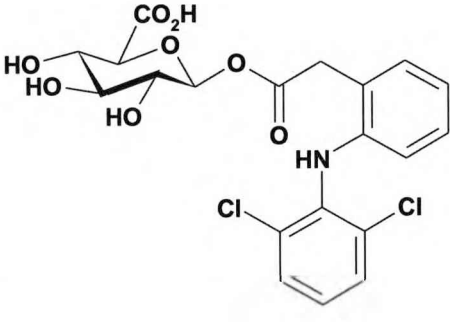
Abstract

Glucuronides are a class of phase II metabolites that are significant in the detoxification of xenobiotics and endogenous compounds. Synthetic samples of glucuronides are important, and can be used as analytical standards, as well as in stability studies in elucidating structure activity relationships with respect to toxicity. The FDA states that if a metabolite is excreted in >10 % of the initial dose, it must be investigated in relation to any adverse drug reactions. Recently we have looked at *O*-acyl (AGs) and *N*-glucuronides.

1-β-*O*-acyl glucuronides (AGs) have long been known to be *reactive* metabolites which can covalently modify body proteins. The consequences are still unclear and it is not certain that AGs are directly toxic. An update on the latest methods of synthesis of AGs will be given, together with insight into the stability of AGs, and their interactions with proteins. The current high concern in the pharmaceutical industry over all reactive metabolites and the occurrence of AGs as major metabolites of common drugs such as Ibuprofen and Diclofenac emphasise the significance of this work.



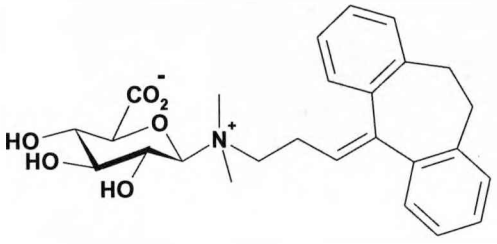
Ibuprofen acyl glucuronide



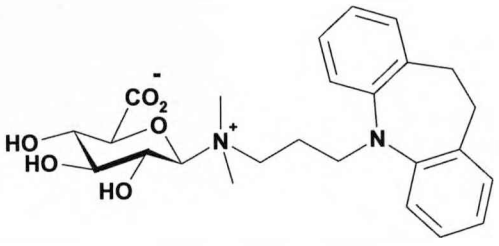
Diclofenac acyl glucuronide

N-Glucuronides have been relatively little studied, but they are important for a number of amine-containing drugs. They are unique human metabolites and are therefore not detected in pre-clinical toxicology. We have lately developed synthetic methods for this class, especially quaternary ammonium glucuronides of aliphatic tertiary amines whose synthesis was previously unsatisfactory.

Valuable information on the acid and base stability of *N*-glucuronides, together with the underlying chemistry of anomeric quaternary ammonium glucosides, has been gathered as a result. Typical examples are the N^+-glucuronides of Amitriptyline and Imipramine, commonly-used tricyclic antidepressants, whose syntheses will be given to illustrate our methodology.



Amitriptyline N^+-Glucuronide



Imipramine N^+-Glucuronide

Publications

Caroline H. Johnson, Ian D. Wilson, John R. Harding, Andrew V. Stachulski, Lisa Iddon, Jeremy K. Nicholson, and John C. Lindon. **NMR Spectroscopic Studies on the in Vitro Acyl Glucuronide Migration Kinetics of Ibuprofen ((±)-(R,S)-2-(4-Isobutylphenyl) Propanoic Acid), Its Metabolites, and Analogues,** Analytical Chemistry, **2007**, 79 (22), 8720-8727

Caroline H. Johnson, Toby J. Athersuch, Ian D. Wilson, Lisa Iddon, Xiaoli Meng, Andrew V. Stachulski, John C. Lindon and Jeremy K. Nicholson. **Kinetic and J-resolved statistical total correlation NMR spectroscopy approaches to structural information recovery in complex reacting mixtures: application to acyl glucuronide intramolecular transacylation reactions**. Analytical Chemistry, **2008**, 80 (13), 4886-95.

Neil G. Berry, Lisa Iddon, Mazhar Iqbal, Xiaoli Meng, Prabha Jayapal, Caroline H. Johnson, Jeremy K. Nicholson, John C. Lindon, John R. Harding, Ian D. Wilson and Andrew V. Stachulski. **A synthetic, NMR and computational study of a set of arylacetic acid 1 β-*O*-acyl glucuronides: a first computational and mechanistic explanation of observed acyl migration rates.** *Accepted by Organic and Biomolecular Chemistry 2009.*

L. Iddon *et al* **Syntheses and structures of anomeric quaternary ammonium β-glucosides: model compounds for zwitterionic N^+-glucuronide drug metabolites,** *accepted by Tetrahedron*

L. Iddon. *et al.* **Synthesis of zwitterionic N^+-glucuronide drug metabolites from the hemiacetal of glucuronic acid,** *in progress*

Acknowledgements

Firstly I would like to thank my supervisors, Dr. Andrew Stachulski, Dr. John Harding and Dr. Ryan Bragg for their help and support throughout my PhD. I would like to thank John Harding and Ryan Bragg for making my placement at AstraZeneca an enjoyable experience.

I would like to thank Xiaoli Meng for her support at the beginning of my PhD, as well as other members of the Stachulski group for making my time in the lab fun as well as a good place to learn.

I am very grateful to the EPSRC and AstraZeneca for funding, without them I would not have the opportunity to work on such an interesting project, in which I have gained invaluable experience.

I would like to thank James Maggs of the department of pharmacology for providing LCMS data. I would also like to thank the technical staff from the department of chemistry for providing elemental analysis, mass spectroscopy, and x-ray crystallography.

To my friends and family I would like to say thank you for the continued support which you have provided during my PhD. I especially want to thank my Mum and Dad for their financial support; without them I would not have been able to continue my horse riding during the last three years.

Abbreviations

$[\alpha]_D^{293K}$	-	Specific rotation at room temperature
ADR	-	Adverse drug reaction
AG	-	Acyl glucuronide
aq	-	aqueous
BAIB	-	Bisacetoxy iodobenzene
CYP	-	Cytochrome P450 monooxygenase
d	-	doublet
DABCO	-	1,4-Diazabicyclo[2.2.2]octane
DAST	-	Diethylaminosulfur trifluoride
DCC	-	N,N'-Dicyclohexyl-carbodiimide
dd	-	doublet of doublets
DIAD	-	Diisopropyl azodicarboxylate
DIC	-	N,N'-Diisopropyl-carbodiimide
DMAP	-	4-Dimethylamino pyridine

DMF	-	N,N'-Dimethylformamide
DMSO	-	Dimethylsulfoxide
D_2O	-	Deuterated water
E2	-	Bimolecular elimination
E1	-	Monomolecular elimination
eq	-	equivalents
g	-	grammes
GA	-	Glucuronic acid
HATU	-	*O*-(7-Azabenzotriazol-1-yl)-N,N,N',N',-Tetramethyluronium hexafluorophosphate
HOBt	-	1-Hydroxybenzotriazole
HPLC	-	High pressure liquid chromatography
HRMS	-	High resolution mass spectroscopy
hr	-	hours
Hz	-	Hertz
IR	-	Infrared

J	-	Coupling constant
LAS	-	Lipase AS amino
LCMS	-	Liquid chromatography mass spectroscopy
m	-	multiplet
ml	-	millilitres
mmol	-	millimoles
mol	-	moles
mp	-	melting point
MHz	-	Megahertz
MS	-	Mass spectroscopy
m/z	-	mass/charge
NMM	-	*N*-methyl morpholine
NMP	-	*N*-methyl pyrrolidine
NMR	-	Nuclear magnetic resonanance
NPI	-	No product isolated
NSAID	-	None steroidal anti-inflammatory drug

$Pd/Pd^{(0)}$	-	Palladium
Pd/C	-	Palladium on charcoal/carbon
PLE	-	Porcine liver esterase
PMB	-	*p*-Methoxybenzyl
ppm	-	parts per million
Py	-	pyridine
q	-	quartet
RT	-	Room temperature
SAR	-	Structure activity relationship
S_N1	-	Monomolecular nucleophilic substitution
S_N2	-	Bimolecular nucleophilic substitution
t	-	triplet
TBAF	-	Tetrabutyl ammonium fluoride
TBDMS	-	*t*Butyl dimethylsilyl
TEMPO	-	2,2,6,6-Tetramethylpiperidine-1-oxyl
TFA	-	Trifluoroacetic acid

THF	-	Tetrahydrofuran
TLC	-	Thin layer chromatography
TMS	-	Trimethylsilyl
UDP	-	Uridine diphosphate
UDPGA	-	Uridine diphosphate glucuronic acid
UDPGT	-	Uridine diphosphate glucuronosyl transferase
w/v	-	weight per 100 ml
σ^*	-	antibonding orbital

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Chapter One

General Introduction

1.1 Drug Metabolism

Metabolism of drugs and other xenobiotics is an important process in all mammals; it is the body's way of eliminating unwanted, often biologically active foreign substances. All food and drugs taken orally and absorbed through the intestinal tract are passed via the portal vein directly to the liver, where the hepatocytes of the liver produce metabolising enzymes that form Phase I and II metabolites[1,2]. There are other sites for the metabolism of xenobiotics including the lungs, kidneys, gut and skin, but the liver is generally considered to be the most important

Phase I Metabolism

Phase I metabolism, generally oxidations, reductions and hydrolytic reactions, involves functional group changes in which small polar groups are added to make the molecule more hydrophilic; or a methyl group removed in order to expose a more polar group, eg -OH, -NH, COOH (figure 1.1.1). The majority of the Phase I oxidative metabolism is mediated by cytochrome P450 enzymes[3].

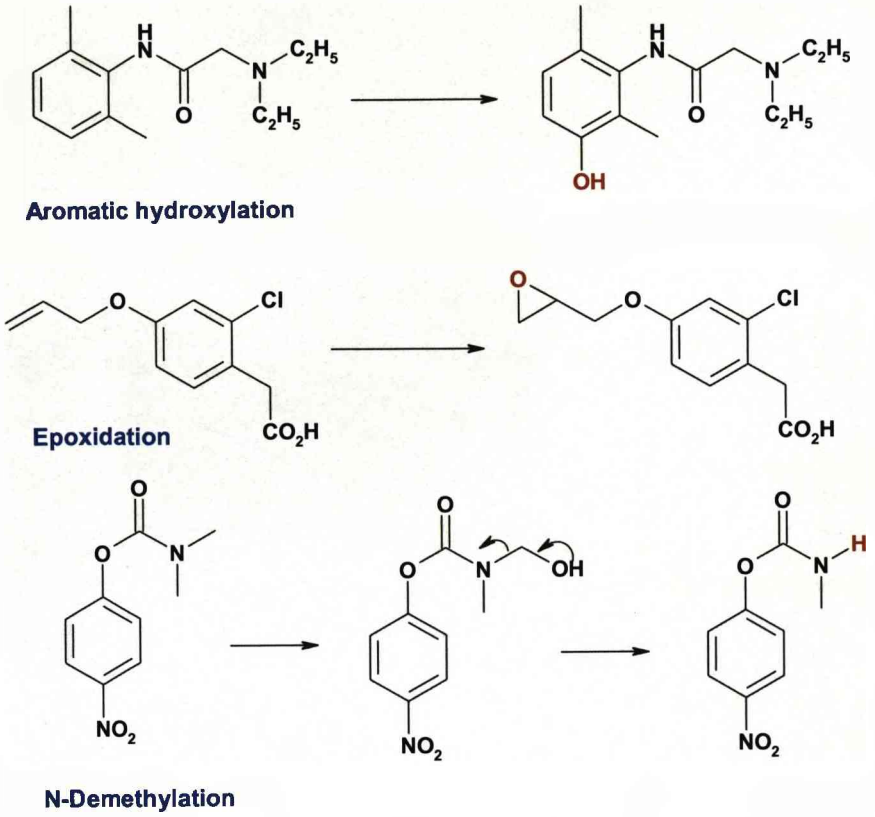
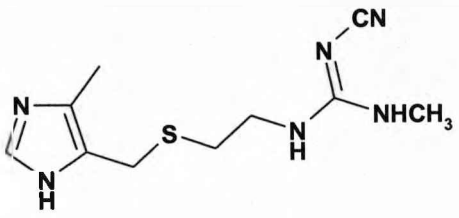
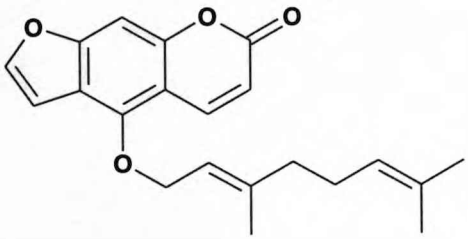


Figure 1.1.1: Some examples of Phase I metabolism[3]

The availability of cytochrome P450 enzymes is controlled by genetics, endogenous hosts and environmental factors[4]. There are some drugs which can inhibit production of metabolites of other drugs via interactions with other CYP enzymes. For example, Cimetidine **(1.1)** was shown to affect drug metabolism in both humans and animal species[5] (figure 1.1.2). Grapefruit juice is known to inhibit intestinal cytochrome P450 enzyme CYP 3A4, which increases the bioavailability of drugs metabolised by this enzyme, leading to overdose effects[6]. This was discovered in 1989, and has been shown to cause deaths due to drug overdose. He *et al.*[7] extracted bergamottin **(1.2)** from grapefruit juice and found that this inhibited CYP 3A4 (figure 1.1.2).



Cimetidine (1.1)



Bergamottin (1.2)

Figure 1.1.2: Compounds found to inhibit Cytochrome P450 enzymes

Phase II Metabolism

During Phase II metabolism a polar group is added, catalysed by transferase enzymes. The addition of polar groups increases the molecule's water solubility and therefore aids excretion. Many metabolites formed during phase I are then able to undergo conjugation during phase II metabolism. The reaction that occurs in Phase II is normally conjugation with; glucuronic acid, sulfates, glutathione or amino acids (figure 1.1.3)[8].

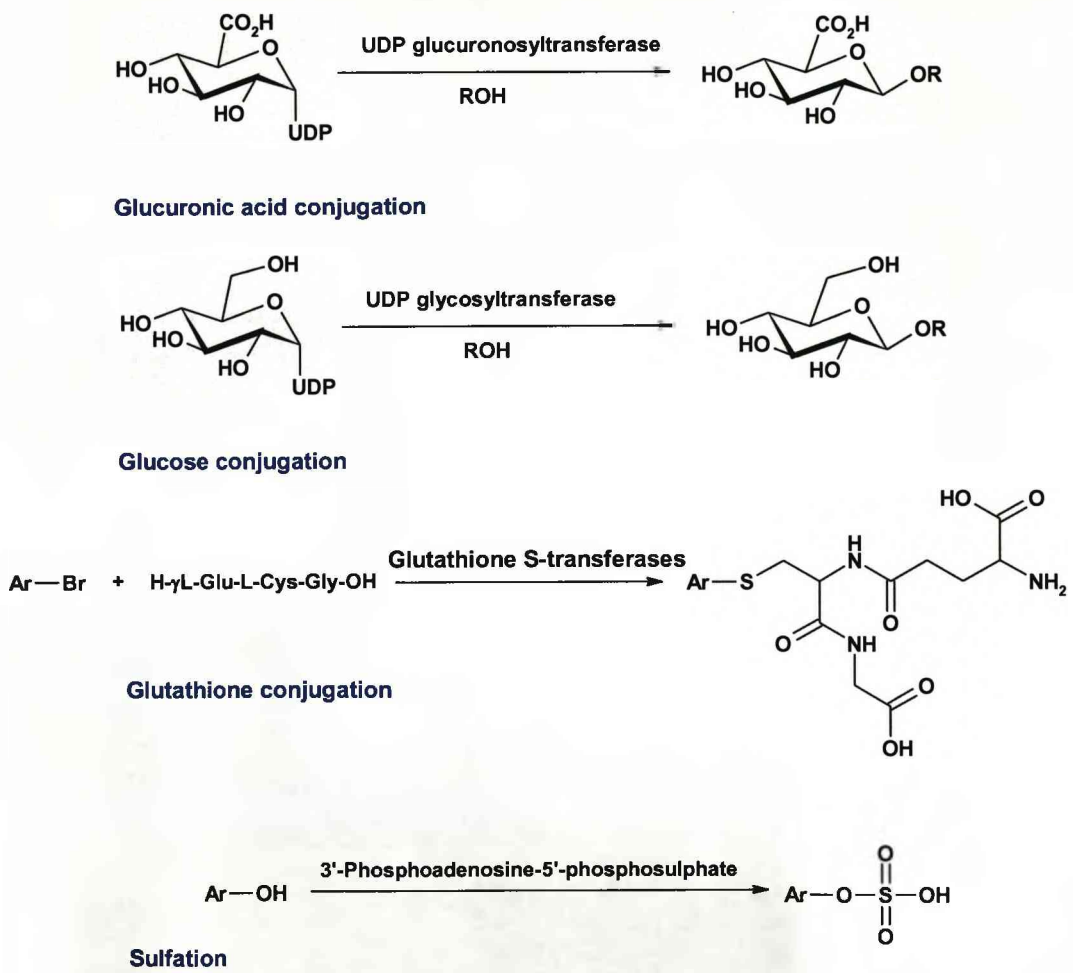
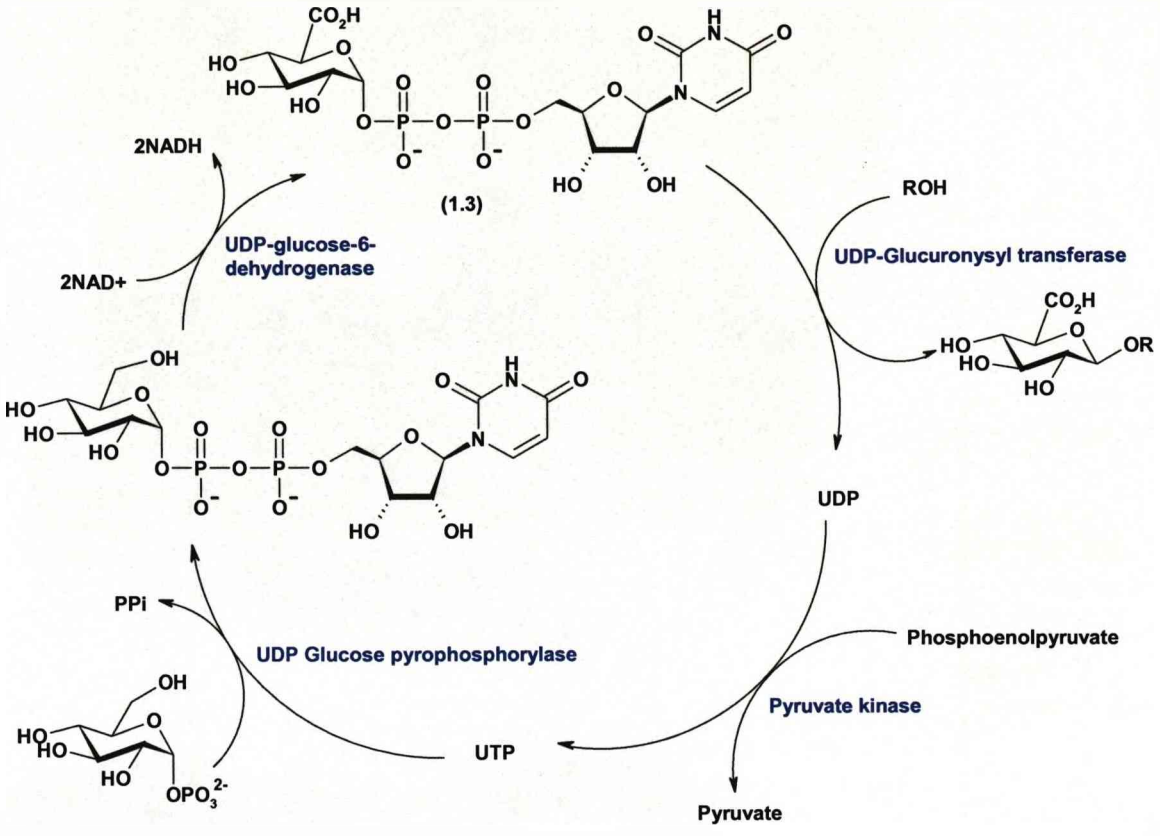


Figure 1.1.3: Most common forms of phase II metabolism

1.2 Glucuronidation

Glucuronidation is the most common type of phase II metabolism for both xenobiotics, (drugs, carcinogens and other environmental pollutants), and of endogenous compounds such as bilirubin and steroids[9]. Glucuronides are formed in the body as a means of reducing biological activity and for the elimination of xenobiotics and endogenous compounds, via the urine, and bile (and hence the faeces). Conjugation with glucuronic acid is via a reactive functional group within the drug molecule. More common reactive functional groups known to undergo glucuronidation are; carboxylic acids, phenols, alkyl alcohols, amines, and thiols.

Glucuronidation in the body occurs via UDP-Glucuronic acid **(1.3)**, this is an S_N2 reaction catalysed by UDP-Glucuronosyltransferase enzymes[10] which are found in many major organs, but predominantely in the liver (scheme 1.2.1). The S_N2 nature of the reaction gives rise to β-configured glucuronides. Once the nucleophilic group has reacted with UDP-Glucuronic acid **(1.3)** to form the glucuronide, UDP is generated, which is then reformed to UDP-GA. The structure of UDP-Glucuronic acid was elucidated by Storey and Dutton in 1955[11].



Scheme 1.2.1: The reaction scheme for the formation of an *O*-glucuronide, and how UDP is reformed to UDP-GA

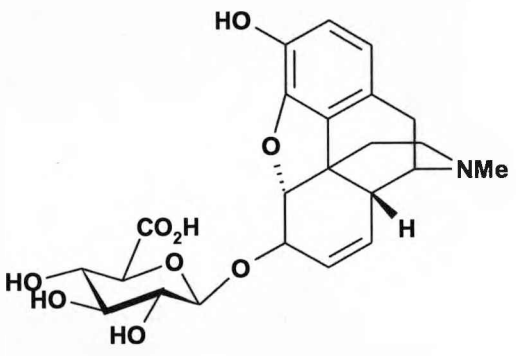
The bond that is formed between the drug and glucuronic acid is known as the glycosidic bond. There are also enzymes that can hydrolyse the glycosidic bond, and these are called glucuronidases.

As part of drug design and development, it is important to understand drug metabolism[1] so that in the future predictive tools may be used. Such predictive tools would then hopefully lead to more informed drug design that will eliminate potential toxic metabolites at an early stage.

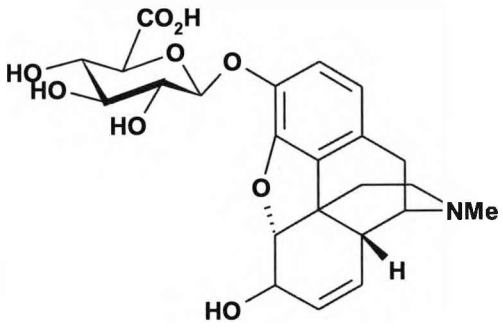
1-β-O-Aryl Glucuronides and O-Alkyl Glucuronides

O-aryl or alkyl glucuronides are formed from a reactive phenol or alcohol group present in the drug molecule. These classes have been known for many years and have been well documented in the literature as metabolites of many drugs[9]. It is believed that generally this class of glucuronide is formed in order to detoxify and deactivate the drug molecule and predominantly has no toxicological implications.

An example of a drug which forms *O*-glucuronides is morphine. There are two sites where glucuronidation occurs; the alkyl alcohol which gives morphine-6-glucuronide (M6G **(1.4)**), and the phenolic alcohol which forms morphine-3-glucuronide (M3G **(1.5)**) (figure 1.2.2). The M6G metabolite is thought to have a higher potency as an analgesic than morphine[12,13-15], whereas M3G is thought to be purely detoxifying. The M6G metabolite is the minor formed, giving a ratio of 1:4 of M6G to M3G.



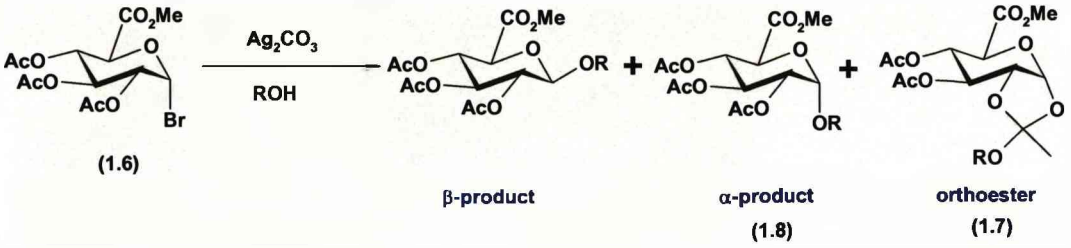
Morphine-6-glucuronide (1.4)



Morphine-3-glucuronide (1.5)

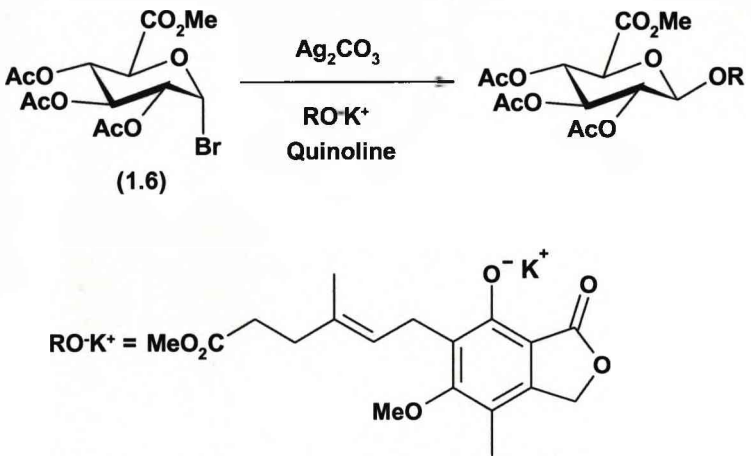
Figure 1.2.2: Morphine-6-*O*-Glucuronide and Morphine-3-*O*-Glucuronide

A typical method used to synthesise *O*-glucuronides is the Koenigs-Knorr reaction. The aglycone is coupled to the sugar activated by anomeric bromine **(1.6)**, which is promoted by Ag(I) salts (scheme 1.2.3)[16]. A disadvantage to this method is the formation of the orthoester **(1.7)** and the undesired α-product **(1.8)** (scheme 1.2.3). A method developed which eliminates the orthoester by-product is the use of anomeric esters, activated by Lewis acids such as $SnCl_4$[17].



Scheme 1.2.3: Koenigs-Knorr reaction, products and by-products

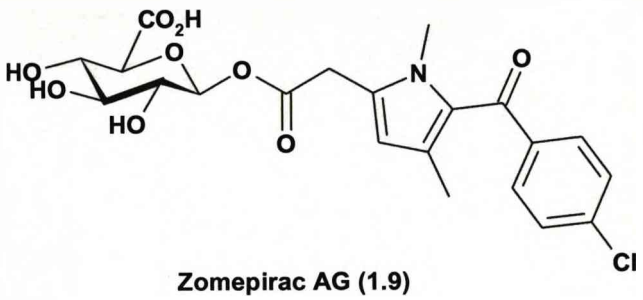
A method typically used for phenols, is the formation of the potassium, sodium or lithium phenolate, which is then reacted with the anomeric bromine **(1.6)**[18]. Ando *et al.* formed the potassium phenolate of mycophenolic acid and used this in their Koenigs-Knorr coupling reaction (scheme 1.2.4)[19].



Scheme 1.2.4: Reaction carried out by Ando *et al.* using the potassium phenolate of mycophenolic acid

1-β-O-Acyl Glucuronides

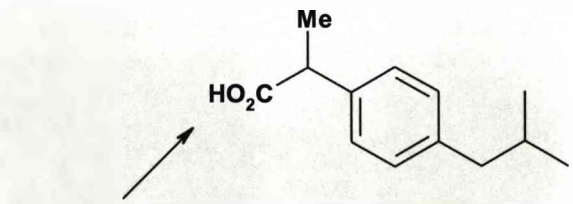
1-β-*O*-acyl glucuronides originate from a drug containing a carboxylic acid group and form an ester bond with glucuronic acid at the anomeric position. It is believed that acyl glucuronides (AGs) are in some cases reactive metabolites which can lead to adverse drug reactions, thought to be due to their interactions with proteins[20]. An example of an acyl glucuronide suspected to form covalent adducts with proteins is zomepirac AG[21] **(1.9)** (figure 1.2.5).



Zomepirac AG (1.9)

Figure 1.2.5

Recently it has been reported[22] that pharmaceutical companies are becoming more wary of potential drug discovery targets which possess a carboxylic acid functional group due to potential AG formation. There are drugs known to form AGs that are relatively safe, such as Ibuprofen **(1.10)** (figure 1.2.6), which indicates more research is required in this field to fully understand why some carboxylic acid containing drugs are 'safe' and some extremely toxic.



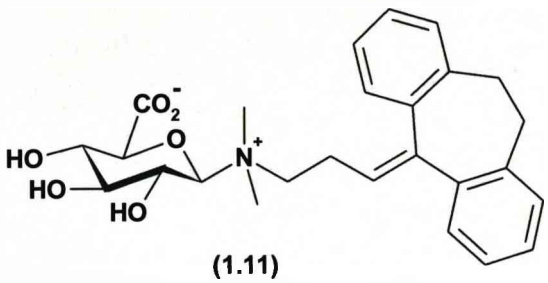
Site of glucuronidation

Figure1.2.6: Ibuprofen **(1.10)** which is dosed as a racemate

See chapter two for more details on AGs.

***N*-Glucuronides and N^+-Glucuronides**

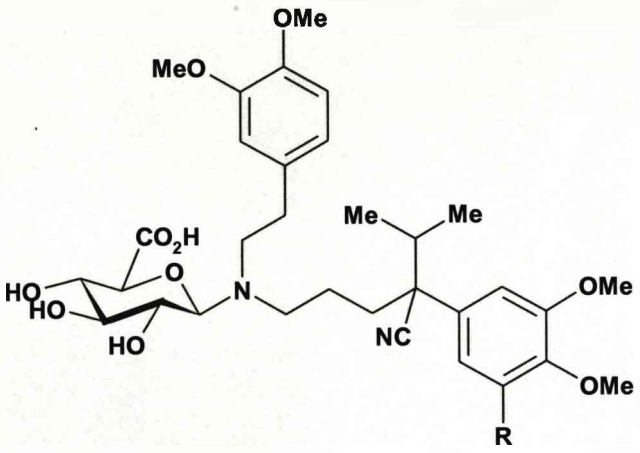
N-glucuronidation has only been discovered relatively recently (~1970s) and is being detected much more frequently for a wide range of drugs[23]. *N*-glucuronidation is common for drugs such as tricyclic antidepressants, antipsychotics and antihistamines which contain a tertiary amine group. Upon glucuronidation these drugs form a zwitterionic species. There are many examples of drugs which have been isolated as their N^+-glucuronide such as Amitriptyline N^+-glucuronide **(1.11)** (figure 1.2.7) which has been isolated in about 12 % of the initial administered dose[24].



(1.11)

Figure 1.2.7: Amitriptyline N^+-Glucuronide

It is known that secondary and primary amines form glucuronides to give the respective neutral species. The *N*-glucuronide of Norgallopamil **(1.12)** and Norverapamil **(1.13)** (scheme 1.2.8) have been isolated as biliary metabolites from rats dosed with the corresponding tertiary amines, Gallopamil and Verapamil[25]. This suggests demethylation during phase I metabolism, followed by *N*-glucuronidation during phase II metabolism.



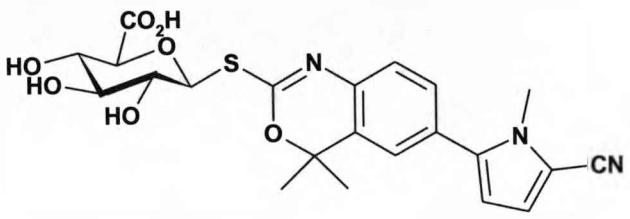
R: OMe; Norgallopamil *N*-Glucuronide (1.12)
H; Norverapamil *N*-Glucuronide (1.13)

Figure 1.2.8

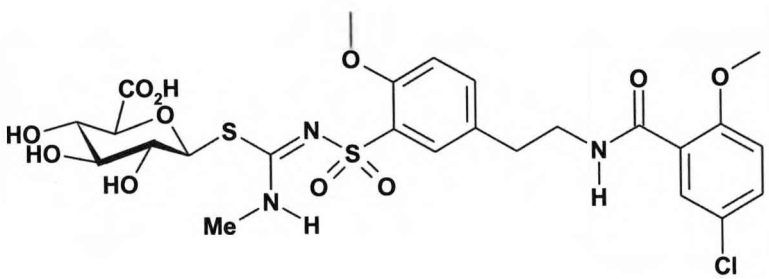
See Chapter three for more details on *N*-glucuronides

1-β-S-Glucuronides

S-Glucuronides are not as common as other types of glucuronides. A recent example of an *S*-glucuronide is HMR1098[26] **(1.14)**, a novel K_{ATP}-blocking agent (figure 1.2.9). Tanaproget-*S*-glucuronide **(1.15)** has been synthesised and characterised by NMR by Keating *et al.*[27] (figure 1.2.9). There have been limited studies of *S*-glucuronides, they are thought to be similar to *O*-glucuronides with respect to toxicity and hydrolysis.



Tanaproget-S-glucuronide (1.15)



HMR1098-S-glucuronide (1.14)

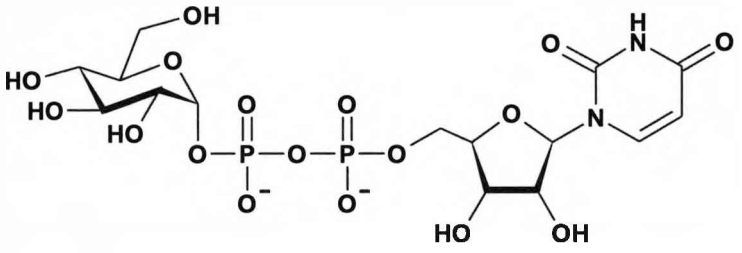
Figure 1.2.9

Dutton *et al.*[28] used in their investigation biosynthetic samples of *o*-aminothiophenol, *p*-nitrothiophenol, diethyldithiocarbamic acid and thiolphenol glucuronides, they concluded that hydrolytically, thiol glucuronides are similar to *O*-glucuronides.

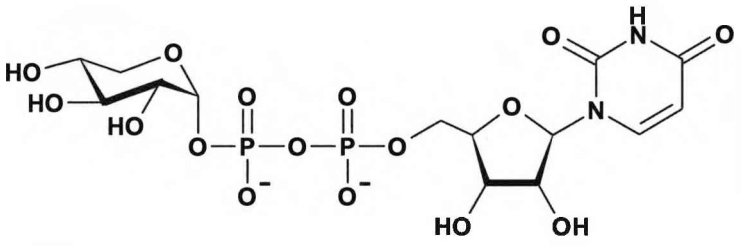
Glycosylation

Conjugation of xenobiotics and endogenous compounds with glucuronic acid is by far the most prevalent type of sugar conjugation, but other sugars are known to undergo conjugation, which include glucose, xylose and ribose[29].

Fevery *et al.*[30] demonstrated that although UDPGA is the primary UDP-sugar co-substrate used for the bilirubin esterification in mammals, two other UDP-sugars; UDP-glucose (UDPG) **(1.16)**, and UDP-xylose **(1.17)**, are also potential co-substrates (figure 1.3.1).



UDP-Glucose (1.16)

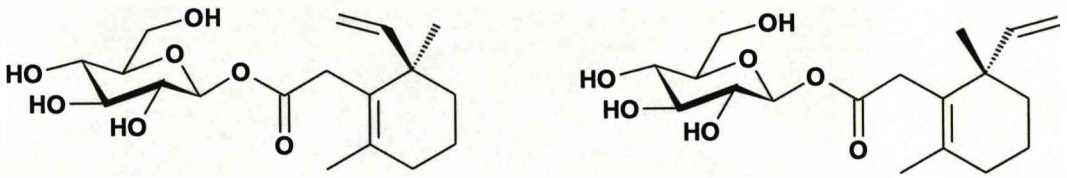


UDP-Xylose (1.17)

Figure 1.3.1

1-β-O-Acyl Glucosides

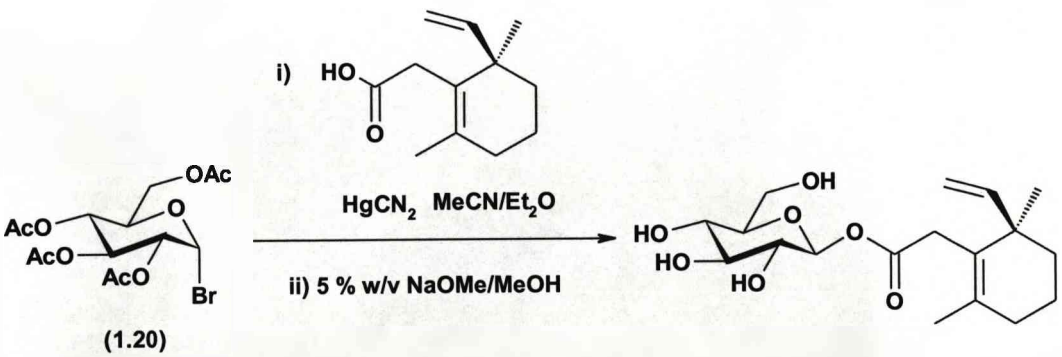
The glycosylation process is found frequently in insects and plants[31], but to a lesser extent in mammals. Walse *et al.*[32] found that the Caribbean fruit fly releases glycoconjugated pheromones named Suspensoside A **(1.18)** and B **(1.19)** (figure 1.3.2).



(6R)-Suspensoside B (1.19) **(6S)-Suspensoside A (1.18)**

Figure 1.3.2: The two diastereoisomeric pheromones produced by the Caribbean fruit fly

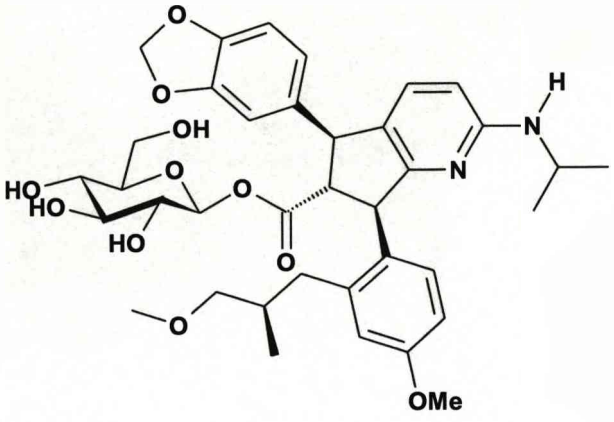
They synthesised both isomers using the bromosugar **(1.20)** as the glycosyl donor, in the presence of Hg (II) (scheme 1.3.3). They then deprotected the acetate groups using sodium methoxide in methanol to give an overall yield of ~35 % (scheme 1.3.3).



(1.20)

Scheme 1.3.3: Conditions used by Walse *et al.* to form the glycosylpheromones, Suspensoside A and B.

A selective and potent endothelin ET_A receptor antagonist underwent significant acyl glucuronidation and acyl glucosidation in human liver microsomes[33] to give **(1.21)** (figure 1.3.4).

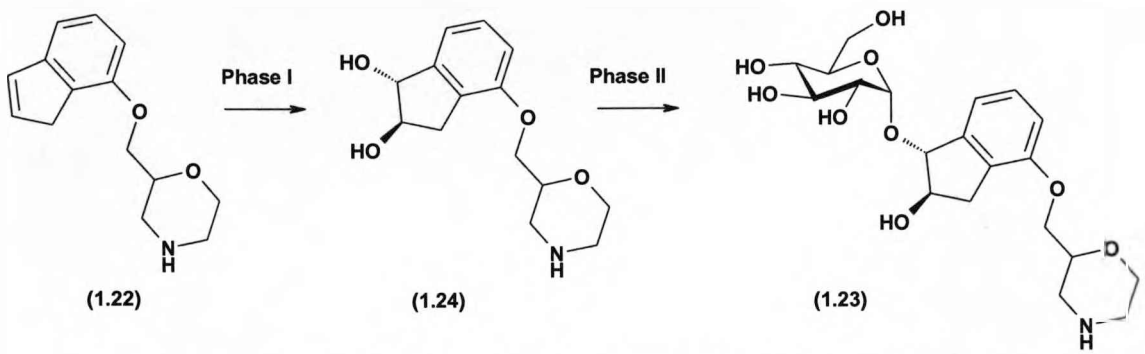


(1.21)

Figure 1.3.4: Example of an acyl glucoside of a selective endothelin ET_A receptor antagonist

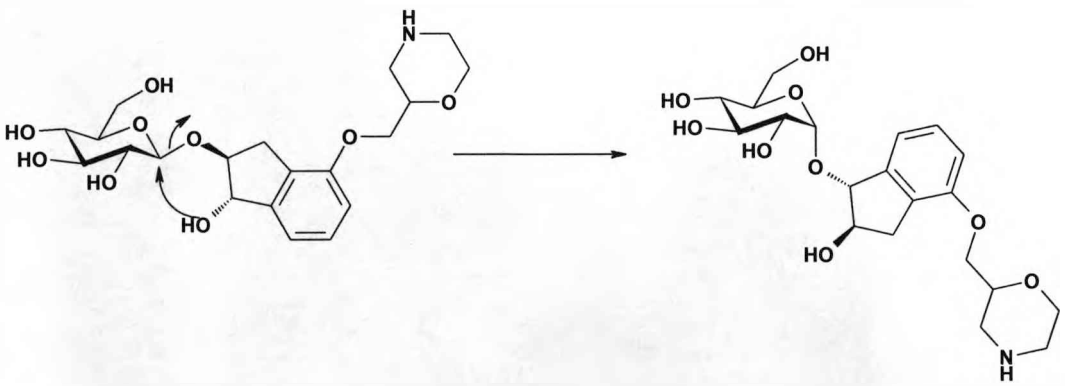
O-Aryl/Alkyl Glucosides

Kamimura *et al.*[34] found that a phase I metabolite of Indeloxazine **(1.22)** formed by rats is excreted as its α-glucoside **(1.23)** (scheme 1.3.5). This is rare, and most glucoside metabolites are found with the β configuration due to the S_N2 reaction with UDP-Glucose.



Scheme 1.3.5: Proposed α-*O*-glucoside of an Indeloxazine phase I metabolite

The diol **(1.24)** formed during phase I metabolism may have a part to play in the formation of the α-glucoside (scheme 1.3.6). Due to the anomeric effect, it would be expected that the α-glucoside would be more thermodynamically stable than the β-glucoside.



Scheme 1.3.6: Possible mechanism for the formation of the α-glucoside

Cervenkova *et al.*[35] carried out investigations to probe the potential of Olomoucine **(1.25)** type derivatives such as Bohemine **(1.26)** (figure 1.3.7) towards glycosylation. They did *in vitro* examinations on mouse, rat, and monkey as well as human liver and kidney microsomes. They found the mouse liver and kidney microsomes contained high levels of bohemine-1-*O*-β-glucoside, but very little was detected in rat, monkey and human microsomes.

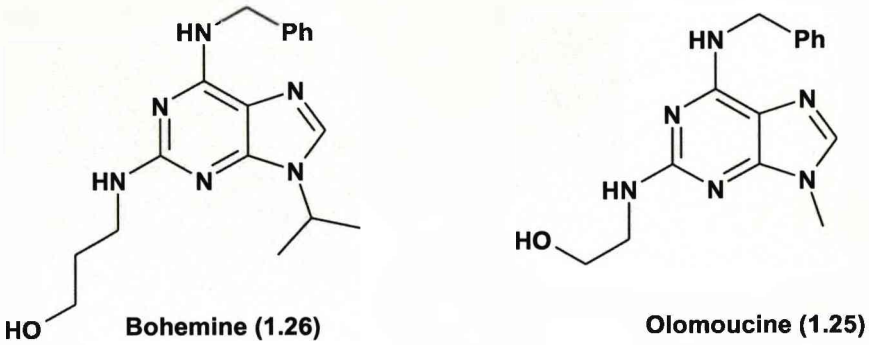
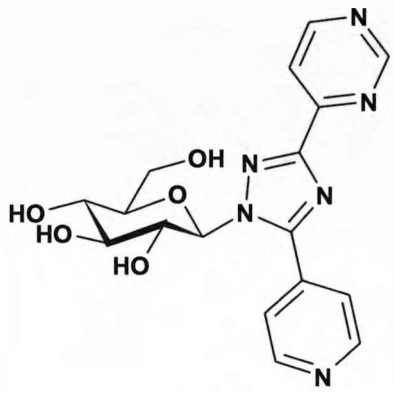


Figure 1.3.7: Bohemine and Olomoucine; two potential candidates for *O*-Glucoside formation

An important point made is that they cannot be certain that conjugation is occurring on the hydroxyl group, there is also potential for *N*-glucosidation to occur.

N-Glucosides

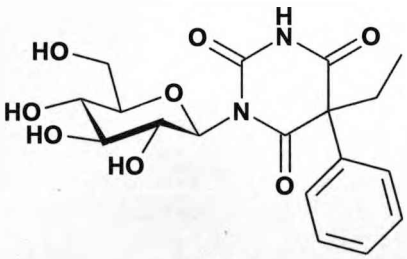
Although rare *N*-glycosylation is known; it is thought to be a detoxification mechanism for xenobiotics. Duggan *et al.*[36] found that a xanthine oxidase inhibitor, forms an *N*-glucoside **(1.27)** via the 1,2,4-triazole ring (figure 1.3.8).



(1.27)

Figure 1.3.8: 3-(4-Pyrimidinyl)-5-(4-pyridyl)-1,2,4-triazole *N*-glucoside

Phenobarbital was found by Tang *et al.*[37] to form an *N*-glucoside **(1.28)** *in vivo*. Two male volunteers were given Phenobarbital. Both metabolised Phenobarbital as an *N*-glucoside **(1.28)** (figure 1.3.9) with the metabolite being isolated from urine in 24 and 30 % of the administered dose.

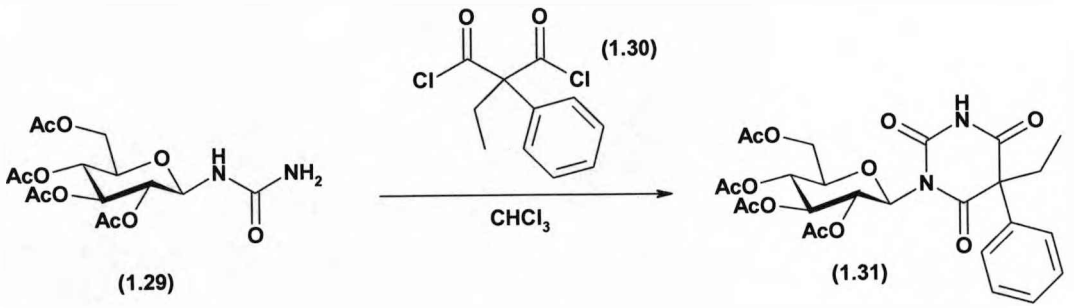


(1.28)

Figure 1.3.9: Phenobarbital *N*-glucoside

Tang *et al.* made a synthetic sample to compare to the *in vivo* sample isolated. The acetylated compound was isolated from the reaction of N-(2,3,4,6-tetraacetyl-1-β-D-glucopyranosyl)urea **(1.29)** with 2-ethyl-2-phenylmalonyl chloride **(1.30)** to give the

product **(1.31)** in 34 % yield (scheme 1.3.10). The product was not deprotected through to the final compound; the reason is not stated but perhaps due to the instability of the compound during deprotection.



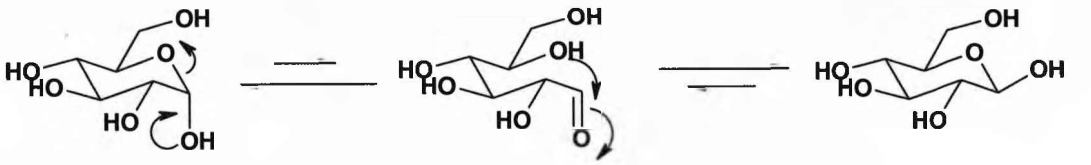
Scheme 1.3.10: Reaction conditions to form the acetylated phenobarbital *N*-Glucoside

1.4 Introduction to Glucose

D-Glucose is a naturally occurring sugar and is the most abundant of its type. It plays a vital role in biochemical transformations such as cellular respiration (Krebs cycle). L-Glucose is not a naturally occurring sugar and cannot be used in biochemical pathways like D-Glucose.

Structure:

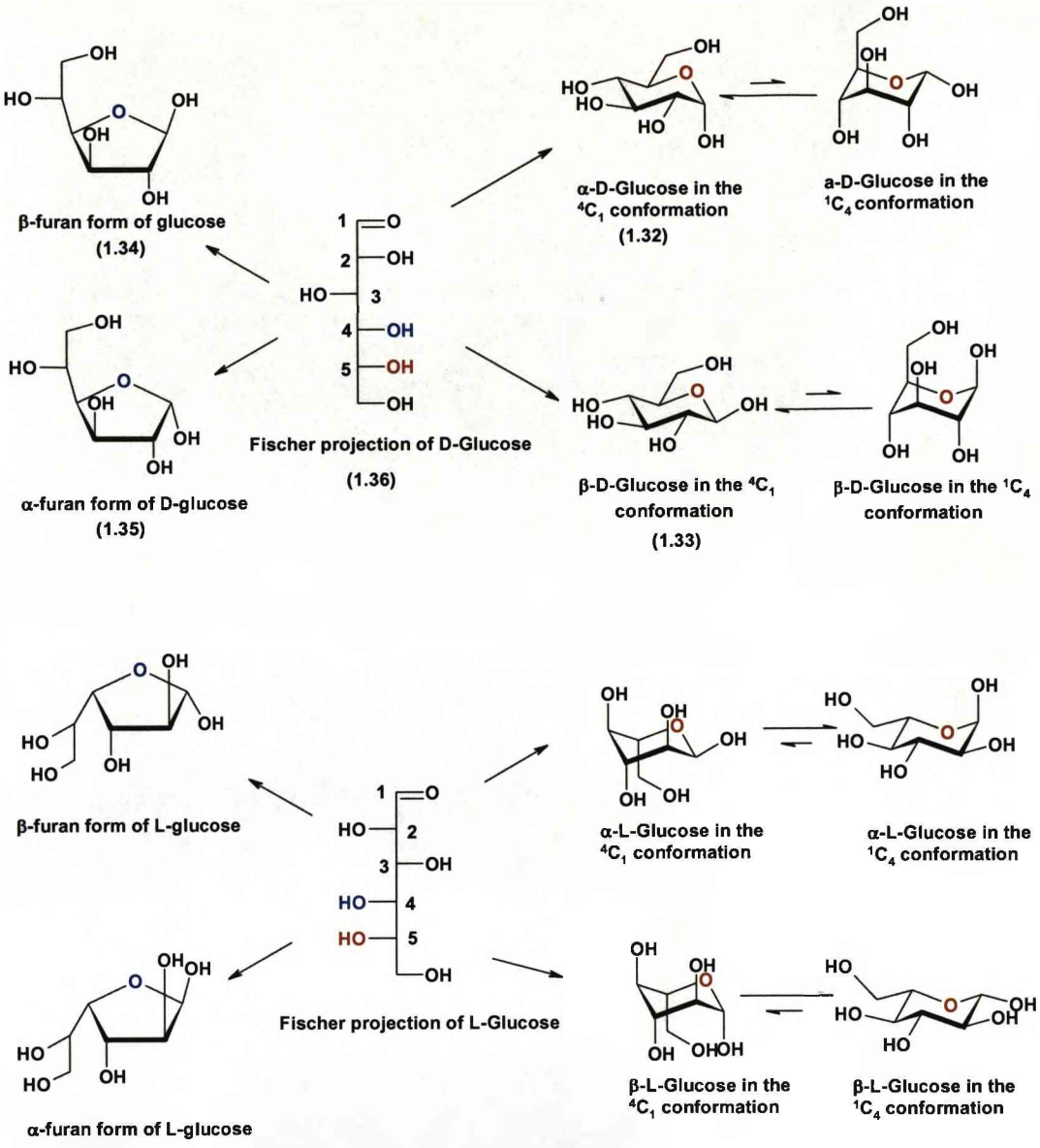
Glucose is known to be most stable as the six membered pyranose, with the ring in the chair conformation. This conformation is favoured in both the solid state and in solution. When glucose is dissolved in D_2O, with immediate measurement by NMR, the spectra shows mainly the α-D-glucose **(1.32)**[38]. On standing in solution the amount of β-D-Glucose **(1.33)** increases until there is approximately a 50:50 mixture of the α and β-anomers which occurs via mutarotation (scheme 1.4.1).



1-α-D-Glucose (1.32) 1-β-D-Glucose (1.33)

Scheme 1.4.1

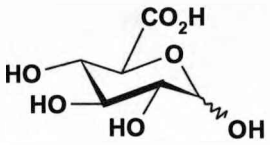
The furanose ring **((1.34)** and **(1.35))** is much less favourable compared to the pyranose ring, and by NMR spectroscopy the furanose is not detected. The acyclic form can be represented by the Fischer projection **(1.36)** (scheme 1.4.2); this schematic shows the open chain aldehyde, but this is unfavoured compared to the six membered hemiacetal. Although the open chain aldehyde is unfavoured it plays a vital role in many reactions.


β-furan form of glucose
(1.34)
α-D-Glucose in the $^4C_1$ conformation
(1.32)
a-D-Glucose in the $^1C_4$ conformation
α-furan form of D-glucose
(1.35)
Fischer projection of D-Glucose
(1.36)
β-D-Glucose in the $^4C_1$ conformation
(1.33)
β-D-Glucose in the $^1C_4$ conformation
β-furan form of L-glucose
α-L-Glucose in the $^4C_1$ conformation
α-L-Glucose in the $^1C_4$ conformation
α-furan form of L-glucose
Fischer projection of L-Glucose
β-L-Glucose in the $^4C_1$ conformation
β-L-Glucose in the $^1C_4$ conformation

Scheme 1.4.2

1.5 Introduction to Glucuronic Acid

D-Glucuronic acid **(1.37)** (figure 1.5.1) is a naturally occurring uronic acid, mainly utilised in metabolism of xenobiotics and bilirubin. In the body the reactive species is UDP Glucuronic acid **(1.3)**; this is synthesised from UDP Glucose by UDP-glucose 6-dehydrogenase (scheme 1.2.1).



Glucuronic acid (1.37)

Figure 1.5.1

Importantly, Glucuronic acid is found copolymerised in a 1:1 ratio with substituted 2-amino-2-deoxy-D-glucose in heparin, chondroitin 4- and 6-sulfates and hyaluronic acid[39].

Heparin's main purpose is thought to be defensive at the site of tissue injury to prevent invasion of bacteria and other foreign materials[40] In medicine it is used as an anticoagulant to prevent deep vein thrombosis (DVT). Heparin is heavily sulfated, and contains repeating units (figure 1.5.2). Another sugar found in heparin is L-iduronic acid, which is the C5 epimer of glucuronic acid.

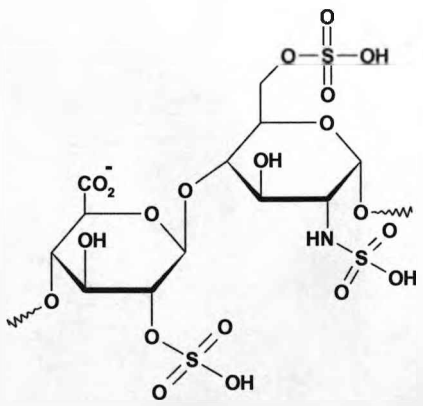


Figure 1.5.2: One of the repeating units found in Heparin sulphate

Hyaluronic acid is found in connective, epithelial and neural tissues. It is a polymer of the disaccharide of D-glucuronic acid and D-N-acetylglucosamine, linked together via alternating β-1,4 and β-1,3 glycosidic bonds (figure 1.5.3).

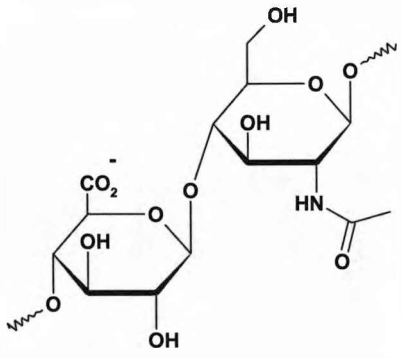
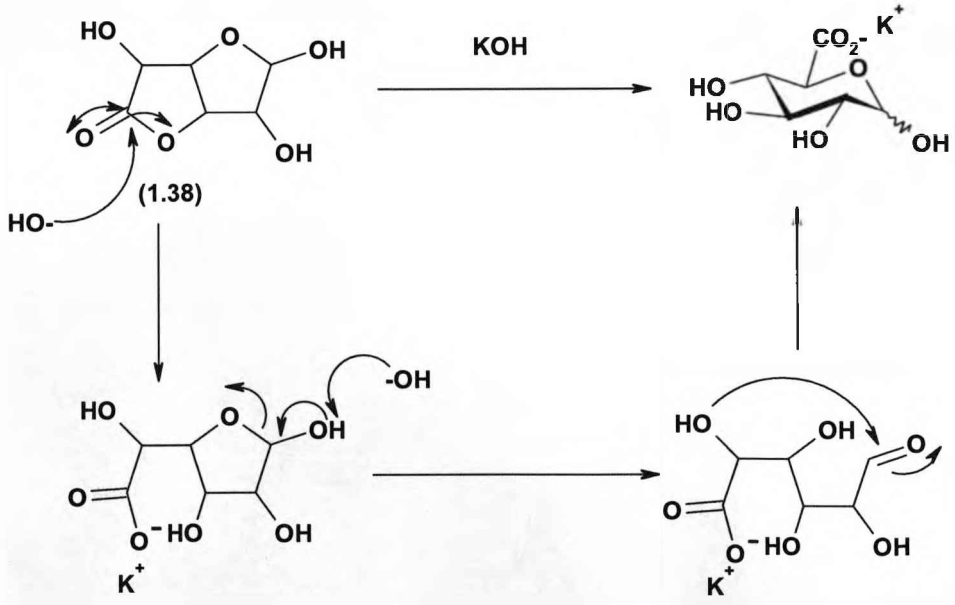


Figure 1.5.3: The β-1,4 repeating unit found in hyaluronic acid

When isolated from aqueous solution, D-glucuronic acid crystallises as monoclinic plates in the glucurono-3,6-lactone form **(1.38)**[39]. The glucuronolactone is hydrolysed readily at high pH to the glucuronate (the sodium or potassium salt) (scheme 1.5.4).



Scheme 1.5.4: Hydrolysis of glucurono-3,6-lactone **(1.38)** to give potassium glucuronate.

Structural studies of glucuronic acid have shown that the potassium salt exhibits the pyranose conformation in which all the hydroxyl groups and the carboxylate ion are in the lower energy equatorial position[41].

1.6 The Anomeric Effect

It is known that cyclohexane rings prefer to have substituents in the equatorial position, with this being the more favoured lower energy orientation of the cyclohexane ring. When it comes to a pyranose ring system, any electronegative substituent at the C1 position prefers to be axial even though sterically this is unfavourable. This was first discovered by Edwards and Lemieux and is now called the Edward Lemieux effect or the anomeric effect[42,43]. There are two main reasons for this phenomenon; the dipole-dipole interaction between the ring oxygen and the electronegative substituent, and the stereoelectronic effect.

The endocyclic sp^3 hybridised oxygen lone pairs form a dipole which points in the exocyclic direction[42]. The dipole-dipole interaction between the endocyclic oxygen and the C1 heteroatom substituent in β-D-Glucose **(1.33)** is unfavourable as they are almost parallel and point in the same direction (figure 1.6.1). The opposite is true for α-D-Glucose **(1.32)** were the dipoles are almost in opposite directions (figure 1.6.1)

β-D-Glucose (1.33) **α-D-Glucose (1.32)**

Figure 1.6.1: Direction of the dipoles in both α and β-glucose

The stereoelectronic effects are due to the lone pairs of the endocyclic oxygen. The axial orientation of the p-orbital that the lone pair occupies is synperiplanar to the antibonding orbital of the anomeric substituent of the α-isomer. It is therefore

possible for the lone pair of the endocyclic oxygen to donate electron density into the σ* orbital, thus stabilising the σ* orbital (figure 1.6.2). This interaction results in a shortening of the O (endocyclic)-C1 bond and a lengthening of the C1-O (exocyclic) bond.

Figure 1.6.2: The σ* orbital is stabilised in the α-conformation

These stabilising effects apply to the $^4C_1$ α-anomer and the $^1C_4$ β-anomer i.e where the substituent is axial (figure 1.6.3) with the $^4C_1$ conformation being the most stable.

$^4C_1$ α-D-Glucose **$^1C_4$ β-D-Glucose**

Figure 1.6.3: The two conformations stabilised by the anomeric effect

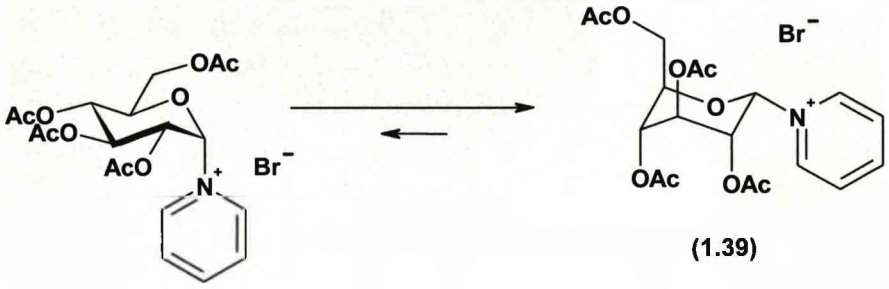
1.7 *The Reverse Anomeric effect (RAE)*

The Reverse Anomeric effect describes the tendency for a positively charged, electronegative anomeric substituent to prefer the equatorial position. Whether the Reverse Anomeric effect exists has been debated, and it has been suggested that it is purely for steric reasons that such substituents prefer the equatorial position[44].

It would be expected that a positively charged substituent would have an enhanced anomeric effect rather than a RAE in terms of the molecular orbital interpretation. A

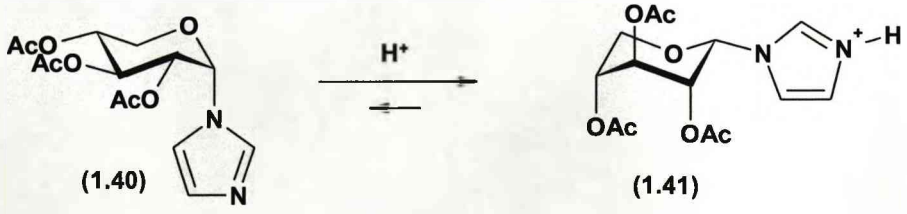
cationic substituent lowers the σ*, which would lead to a stronger interaction between the oxygen lone pair HOMO and the σ* orbital of the C-X^+ bond.

The first example where a RAE was postulated was the *N*-(α-glycosyl)-pyridinium ion **(1.39)**[45,46,47]. These results have been questioned as to whether the effect is due simply to steric repulsions of the axial pyridinium ion (scheme 1.7.1).



Scheme 1.7.1: Pyridinium species thought to exhibit the RAE

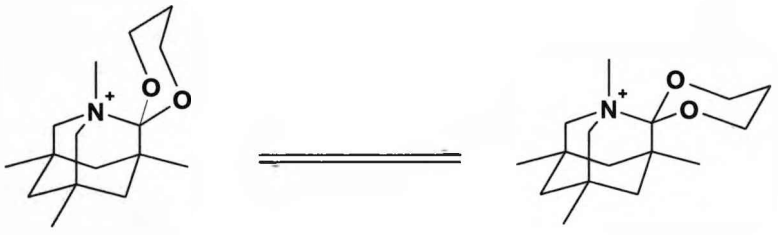
Another example which is thought to exhibit the RAE is N-(tri-*O*-acetyl-α-D-xylopyranosyl)imidazole, where there is a shift from the axial $^4C_1$ conformer **(1.40)** to the equatorial $^1C_4$ conformer **(1.41)** (> 95 %) upon protonation with trifluoroacetic acid[47] (scheme 1.7.2). The ratios before protonation of the two conformers $^4C_1$:$^1C_4$ are 65:35, and it is thought that protonation does not affect steric bulk. It has been said that the shift in equilibrium is due to possible solvation effects, which effectively increase the substituent size[44].



Scheme 1.7.2

Perrin *et al.*[48] have shown in NMR titration studies using *d*-trifluoroacetic acid that the the equilibrium is actually shifted towards the α-anomer, showing a normal anomeric effect.

Jones and Kirby *et al.*[49] came to the conclusion that such an effect does not exist during their studies. They used 1,3-dioxane as a substitute for the pyranose ring system, and a azaadamante substituent (figure 1.7.3). They believe that the two conformers are almost sterically neutral with a slight preference for **(1.43)** over **(1.42)** due to the shorter bond length of N^+-CH_3 compared to C-CH_3 (**(1.43)** is favoured by RAE). They actually found that a conformer close to **(1.42)** was preferred in solution.



(1.42) **(1.43)**

Figure 1.7.3: The two possible conformers, with **(1.42)** being preferred in solution

1.8 *References*

(1) Rostami-Hodjegan, A.; Tucker, G. T. *Nature Reviews Drug Discovery* **2007**, *6*, 140-148.

(2) Thomas, G. *Medicinal Chemistry*, 2007; Vol. 2nd Edition.

(3) Ionescu C, C. M. R. *Drug Metabolism, current concepts*; Springer Netherlands 2005.

(4) Pelkonen, O.; Turpeinen, M.; Hakkola, J.; Honkakoski, P.; Hukkanen, J.; Raunio, H. *Archives of Toxicology* **2008**, *82*, 667-715.

(5) Rendic, S.; Sunjic, V.; Toso, R.; Kajfez, F.; Ruf, H. H. *Xenobiotica* **1979**, *9*, 555-564.

(6) Bailey, D. G.; Malcolm, J.; Arnold, O.; Spence, J. D. *British Journal of Clinical Pharmacology* **1998**, *46*, 101-110.

(7) He, K.; Iyer, K. R.; Hayes, R. N.; Sinz, M. W.; Woolf, T. F.; Hollenberg, P. F. *Chemical Research in Toxicology* **1998**, *11*, 252-259.

(8) Mulder, G. J. *Conjugation reactions in drug metabolism; an integral approach*; Taylor and Francis.

(9) Ritter, J. K. *Chemico-Biological Interactions* **2000**, *129*, 171-193.

(10) Miners, J. O.; Mackenzie, P. I. *Pharmacology & Therapeutics* **1991**, *51*, 347-369.

(11) Storey, I. D. E.; Dutton, G. J. *Biochemical Journal* **1955**, *59*, 279-288.

(12) Yoshimur.H; Oguri, K.; Tsukamot.H *Biochemical Pharmacology* **1969**, *18*, 279.

(13) Paul, D.; Standifer, K. M.; Inturrisi, C. E.; Pasternak, G. W. *Journal of Pharmacology and Experimental Therapeutics* **1989**, *251*, 477-483.

(14) Osborne, R.; Joel, S.; Trew, D.; Slevin, M. *Clinical Pharmacology & Therapeutics* **1990**, *47*, 12-19.

(15) Osborne, R.; Joel, S.; Trew, D.; Slevin, M. *Lancet* **1988**, *1*, 828-828.

(16) Kaspersen, F. M.; Vanboeckel, C. A. A. *Xenobiotica* **1987**, *17*, 1451-1471.

(17) Honma, K.; Nakazima, K.; Uematsu, T.; Hamada, A. *Chemical & Pharmaceutical Bulletin* **1976**, *24*, 394-399.

(18) Yoshida, K.; Iino, N.; Koga, I. *Chemical & Pharmaceutical Bulletin* **1975**, *23*, 1759-1763.

(19) Ando, K.; Suzuki, S.; Arita, M. *Journal of Antibiotics* **1970**, *23*, 408.

(20) Kauffman, F. C. *Conjugation-Deconjugation Reactions in Drug Metabolism and Toxicity*; Springer-Verlag, Berlin, 1994.

(21) Hasegawa, J.; Smith, P. C.; Benet, L. Z. *Drug Metabolism and Disposition* **1982**, *10*, 469-473.

(22) Walker, G. S.; Atherton, J.; Bauman, J.; Kohl, C.; Lam, W.; Reily, M.; Lou, Z.; Mutlib, A. *Chemical Research in Toxicology* **2007**, *20*, 876-886.

(23) Hawes, E. M. *Drug Metabolism and Disposition* **1998**, *26*, 830-837.

(24) Breyer-Pfaff, U. *Drug Metabolism Reviews* **2004**, *36*, 723-746.

(25) Mutlib, A. E.; Nelson, W. L. *Journal of Pharmacology and Experimental Therapeutics* **1990**, *252*, 593-599.

(26) Ethell, B. T.; Riedel, J.; Englert, H.; Jantz, H.; Oekonomopulos, R.; Burchell, B. *Drug Metabolism and Disposition* **2003**, *31*, 1027-1034.

(27) Keating, K. A.; McConnell, O.; Zhang, Y. R.; Shen, L.; DeMaio, W.; Mallis, L.; Elmarakby, S.; Chandrasekaran, A. *Drug Metabolism and Disposition* **2006**, *34*, 1283-1287.

(28) Dutton, G. J.; Illing, H. P. A. *Biochemical Journal* **1972**, *129*, 539.

(29) G. Gibson, P. S. *Introduction to drug metabolism*; 3rd Edition ed.

(30) Fevery, J.; Vandevijver, M.; Michiels, R.; Heirwegh, K. P. M. *Biochemical Journal* **1977**, *164*, 737-746.

(31) Klinotova, E.; Krecek, V.; Klinot, J.; Endova, M.; Eisenreichova, J.; Budesinsky, M.; Sticha, M. *Collection of Czechoslovak Chemical Communications* **1997**, *62*, 1776-1798.

(32) Walse, S. S.; Lu, F.; Teal, P. E. A. *Journal of Natural Products* **2008**, *71*, 1726-1731.

(33) Tang, C. Y.; Hochman, J. H.; Ma, B.; Subramanian, R.; Vyas, K. P. *Drug Metabolism and Disposition* **2003**, *31*, 37-45.

(34) Kamimura, H.; Ogata, H.; Takahara, H. *Drug Metabolism and Disposition* **1992**, *20*, 309-315.

(35) Cervenkova, K.; Belejova, M.; Chmela, Z.; Rypka, M.; Riegrova, D.; Michnova, K.; Michalikova, K.; Surova, I.; Brejcha, A.; Hanus, J.; Cerny, B.; Fuksova, K.; Havlicek, L.; Vesely, J. *Physiological Research* **2003**, *52*, 467-474.

(36) Duggan, D. E.; Baldwin, J. J.; Arison, B. H.; Rhodes, R. E. *Journal of Pharmacology and Experimental Therapeutics* **1974**, *190*, 563-569.

(37) Tang, B. K.; Kalow, W.; Grey, A. A. *Drug Metabolism and Disposition* **1979**, *7*, 315-318.

(38) Ferrier, R. and Collins, P. *Monosaccharides*. Their chemistry and their role in natural products. Wiley and sons, 1995

(39) Dutton, G. J. *Glucuronic Acid, free and condensed*. 1996

(40) Nader, H. B.; Chavante, S. F.; dos-Santos, E. A.; Oliveira, F. W.; de-Paiva, J. F.; Jeronimo, S. M. B.; Medeiros, G. F.; de-Abreu, L. R. D.; Leite, E. L.; de-Sousa, J. F.; Castro, R. A. B.; Toma, T.; Tersariol, I. L. S.; Porcionatto, M. A.; Dietrich, C. P. In *5th Brazilian Symposium on Extracellular Matrix (SIMEC 98)* Angra Dos Reis, Brazil, 1998, p 529-538.

(41) Gurr, G. E. *Acta Crystallographica* **1963**, *16*, 690.

(42) Levy, D. E. *The Organic Chemistry of Sugars*; CRC Press, 2006.

(43) Juaristi, E.; Cuevas, G. *Tetrahedron* **1992**, *48*, 5019-5087.

(44) Perrin, C. L.; Armstrong, K. B. *Journal of the American Chemical Society* **1993**, *115*, 6825-6834.

(45) Dauben, W. G.; Kohler, P. *Carbohydrate Research* **1990**, *203*, 47-56.

(46) Lemieux, R. U.; Morgan, A. R. *Canadian Journal of Chemistry* **1965**, *43*, 2205.

(47) Paulsen, H.; Gyorgyde.Z; Friedman.M *Chemische Berichte-Recueil* **1974**, *107*, 1590-1613.

(48) Perrin, C. L.; Fabian, M. A.; Brunckova, J.; Ohta, B. K. *Journal of the American Chemical Society* **1999**, *121*, 6911-6918.

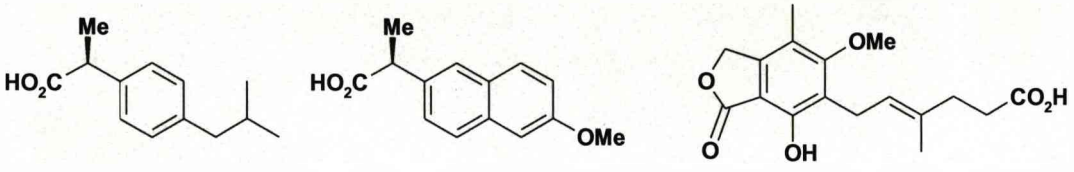
(49) Jones, P. G.; Kirby, A. J.; Komarov, I. V.; Wothers, P. D. *Chemical Communications* **1998**, 1695-1696.

Chapter Two

1-β-O-Acyl Glucuronides

2.1 Introduction to 1β-O-Acyl Glucuronides

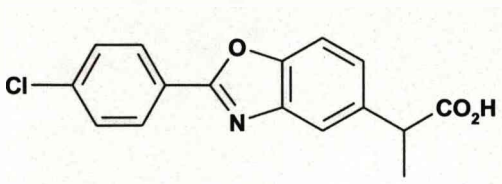
1-β-*O*-Acyl glucuronides are phase II metabolites formed from a drug molecule possessing a reactive carboxylic acid moiety. There have been many carboxylic acid containing drugs isolated as their acyl glucuronide, such as *(S)*-ibuprofen **(1.10)**[1], *(S)*-naproxen **(2.1)**, and mycophenolic acid **(2.2)**[2] (figure 2.1.1).



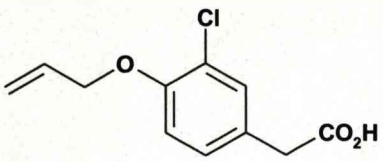
Ibuprofen (1.10) **Naproxen (2.1)** **Mycophenolic Acid (2.2)**

Figure 2.1.1: Carboxylic acid containing drugs.

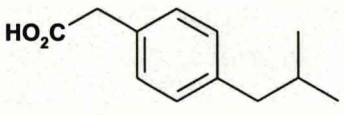
In some cases the acyl glucuronide can be active itself against the receptor or enzyme target; these are called active metabolites. Acyl glucuronides are also known as reactive metabolites due to their ability to covalently bind to proteins; these modified proteins can lead to Adverse Drug Reactions (ADRs). Many drugs removed from market due to toxicity have been carboxylic acids, known to form acyl glucuronides[3]. Benoxaprofen **(2.3)**, Alcofenac **(2.4)**, Ibufenac **(2.5)** and Tienilic acid **(2.6)** (figure 2.1.2) all form acyl glucuronides, and all have been removed from the market due to toxicological implications. There is no evidence that implicates solely the acyl glucuronide of each drug being the reason for such adverse drug reactions, and more investigations are required.



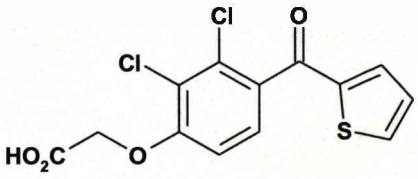
Benoxaprofen
(2.3)



Alcofenac
(2.4)



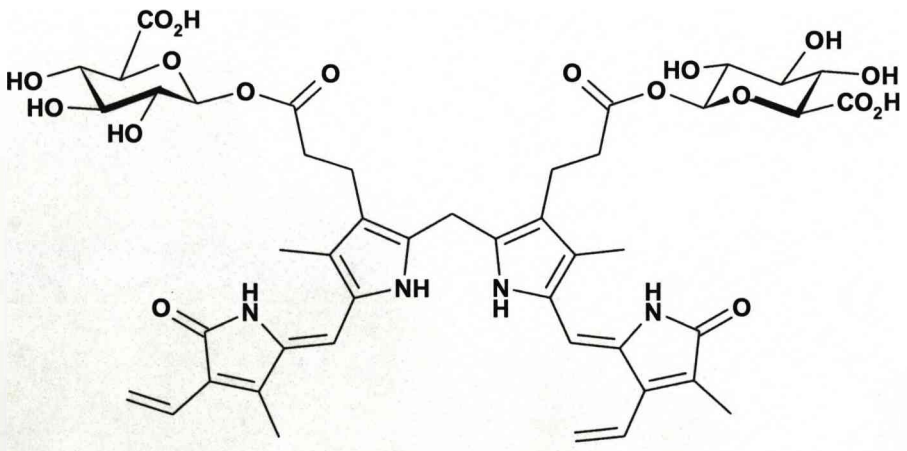
Ibufenac
(2.5)



Tienilic acid
(2.6)

Figure 2.1.2

Acyl glucuronides of endogenous substances are known such as bilirubin diglucuronide **(2.7)** (figure 2.1.3). Bilirubin originates from the breakdown of heme in red blood cells; without removal a build up can lead to jaundice. The conjugation of two glucuronic acid molecules to bilirubin increases its aqueous solubility and hence aids excretion via the faeces.



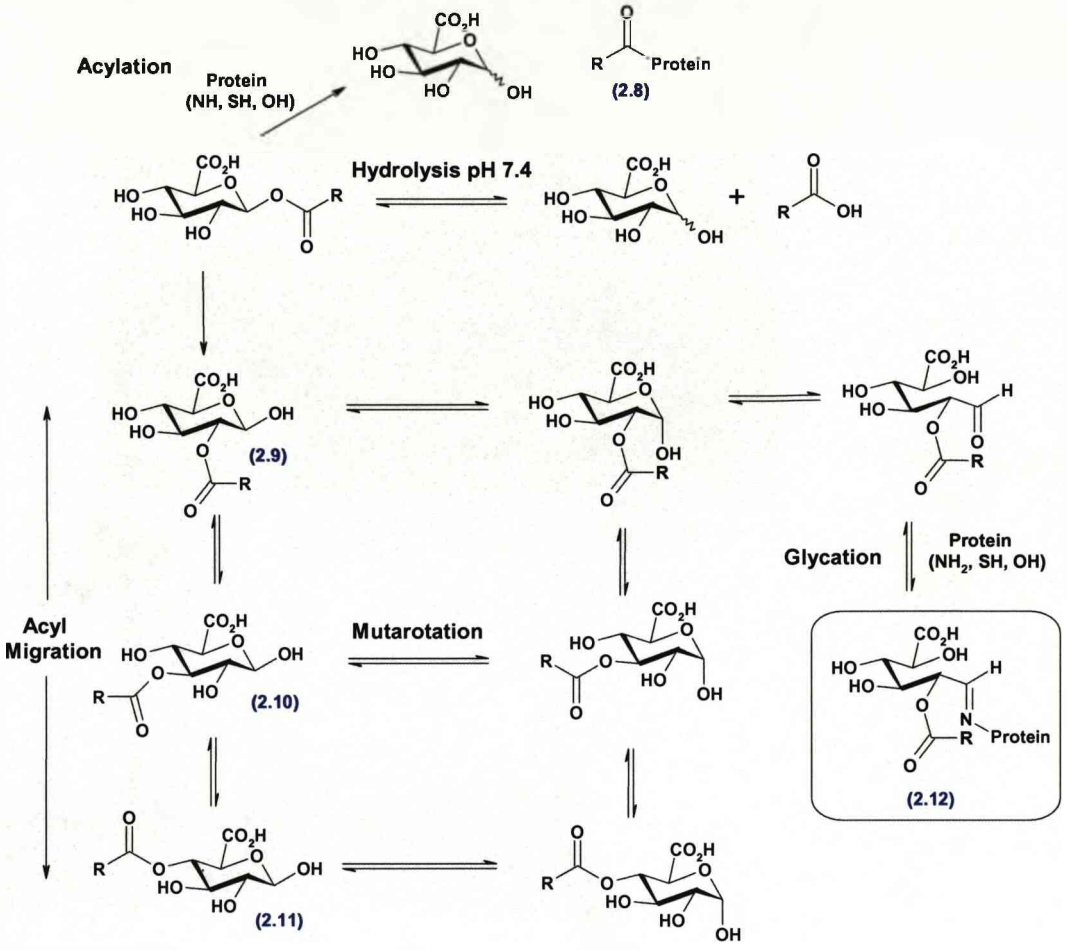
(2.7)

Figure 2.1.3: Bilirubin diglucuronide

2.2 The Reactivity of 1-β-O-Acyl Glucuronides

The electrophilc nature of the acyl glucuronide leads to substitution reactions with nucleophilic molecules in proteins such as -NH_2, -SH and -OH groups. The protein may become directly acylated **(2.8)** and is therefore modified by the drug molecule[4] (scheme 2.2.1).

Acyl glucuronides can also migrate between adjacent hydroxyl groups resulting in 2-*O*-**(2.9)**, 3-*O*-**(2.10)** and 4-*O*-**(2.11)** isomers which are not readily hydrolysed by β-glucuronidase. Once migration has occurred only the 1-α-acyl glucuronide can be formed on the ester group returning to the anomeric position[5]. The 2-*O*-, 3-*O*- and 4-*O*- isomers can undergo mutarotation via the aldehyde form of the sugar resulting in α and β isomers of each migratory product (scheme 2.2.1). The migration is pH dependent occurring at neutral to slightly alkaline environments[2]. Once migration has occurred, the acyl group in the 2-, 3-, and 4-position is less reactive towards nucleophilic attack by proteins. The open chain aldehyde form of the sugar is able to react with nucleophilic groups within proteins, which again leads to a modified protein **(2.12)**; this process is called glycation (Scheme 2.2.1).



Scheme 2.2.1: Pathways which can occur in the body once the acyl glucuronide is formed

Quantitative structure-activity relationships (QSAR's) have been studied by Vanderhoeven *et al.*[6] to establish if there is relationship between the structure of the acyl glucuronide and its ability to migrate or hydrolyse. The degradation rate (k_d) is a combination of the hydrolysis and migration; in general acyl migration is dominant *in vitro*. The reaction rate is followed by ^{1}H NMR, looking at the disappearance of the anomeric proton of the 1β-*O*-acyl isomer as the reaction progresses in the NMR tube under physiological conditions.

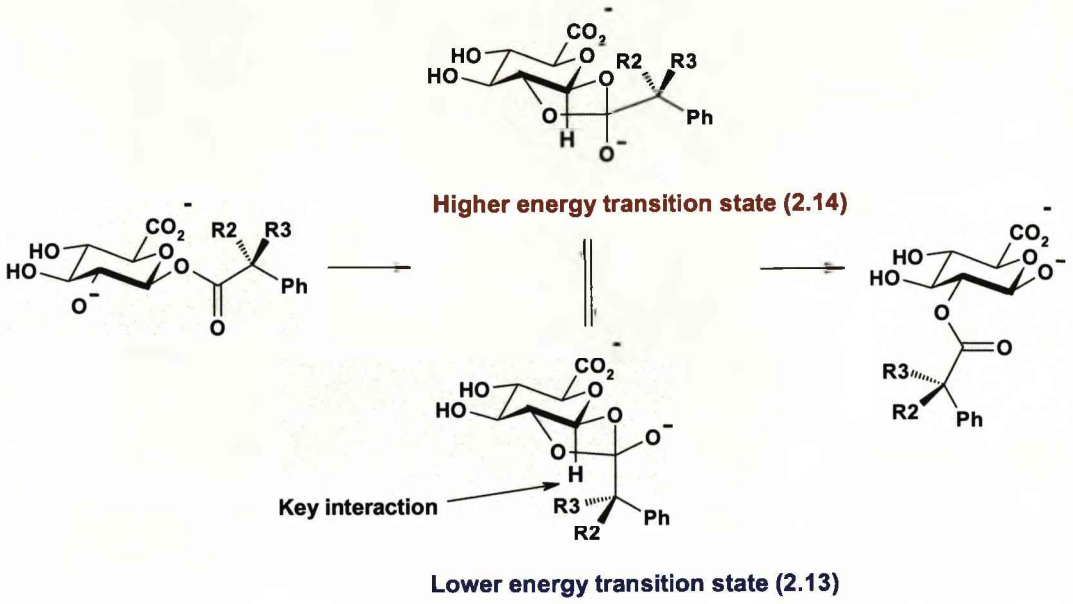
They studied various AGs of para-substituted benzoic acids. They found a good correlation between the Hammett constant (σ_p) and degradation rate ((log)k_d) which

indicates the nucleophilic nature of the reaction. They found that $\rho = +1.63$ indicating the build up of negative charge during the reaction.

There have been reported differences in acyl migration rates between diasteriomeric AGs, such as the AGs of *(R)*- and *(S)*- Ibuprofen **(1.10)**[7]. It has been shown that both isomers hydrolyse at the same rate; therefore the difference in k_d must be due to migration. The *(R)*-isomer (k_d 0.387 h^{-1}) migrates at a faster rate than the *(S)*-isomer (k_d 0.188 h^{-1}). There have been several postulated reasons for this, one being steric effects. It has been suggested that the distance between the aglycone at C1 and the attacking 2-alkoxide nucleophile is greater for the (*S)*-isomer and therefore leads to slower migration[8].

A study carried out by Hasegawa[9] on the AGs of *(R)* and *(S)*-2-phenyl propionoic acid, found that the free energy of activation ΔG for the transacylation of the *(R)*-isomer was smaller than that for the *(S)*-isomer, therefore the *(R)*-isomer would be more reactive.

Molecular modelling calculations carried out by Berry *et al.*[10] on *(R)//(S)*-2-phenyl propionoic acid derived AGs showed that the difference in rates of migration can be explained by the transition states of the ortho ester. From calculations it is believed that in the lower energy transition state **(2.13)** (scheme 2.2.2) if R_3 is methyl (*(S)*-isomer) then the interaction between the axial anomeric hydrogen and the methyl group are significant enough to force the *(S)*-isomer into the higher energy transition state **(2.14)**.



Scheme 2.2.2: The orthoester transition states for the migration of the acyl group

A difference in isomer reactivity has also been observed in protein binding studies. Bischer *et al.*[11] found that *(R)*-naproxen AG had a stronger affinity for protein binding compared to *(S)*-naproxen AG (*(S)*-Naproxen **(2.1)**).

It has in general been found that the more substituted the α-carbon, the slower the rate of migration. NMR studies carried out by Johnson[10] showed that increasing α-methyl substitution of phenyl acetic acid AGs slowed down migration and hydrolysis. Phenylacetic acid AG **(2.15)** had the fastest k_d of 2.353 h^{-1}, methylation at the α-carbon slowed the degradation to 0.903 h^{-1} and 0.405 h^{-1} for the (R)- **(2.16)** and (S)- **(2.17)** mono-α-methyl phenylacetic acid AGs respectively and 0.029 h^{-1} for the di-α-methyl phenylacetic acid AG **(2.18)** (figure 2.2.3).

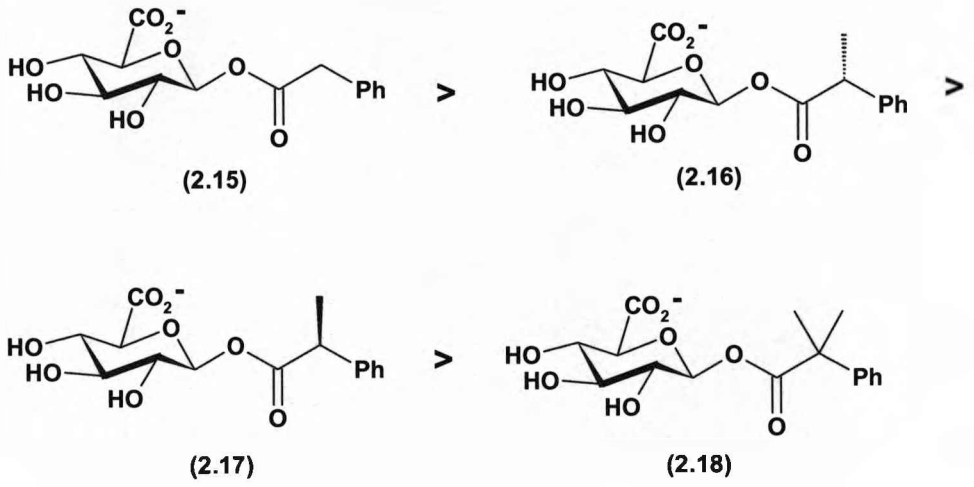
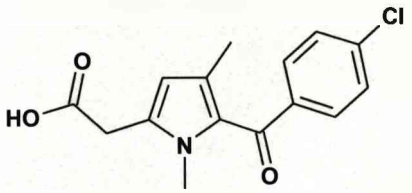


Figure 2.2.3: Increased α-substituted phenyl acetic acid AGs, from left to right migration slows down.

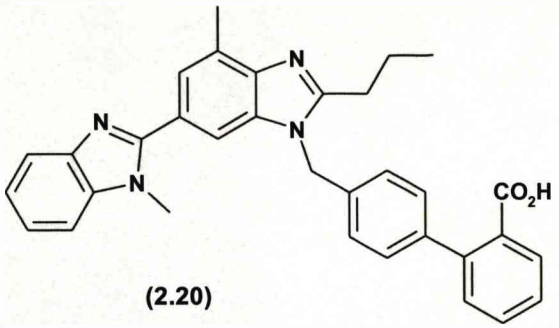
2.3 Stability of 1-β-O-Acyl Glucuronides in Physiological media

The stability of acyl glucuronides in physiological media has been studied; an example of which is Zomepirac[12] **(2.19)** (figure 2.3.1). Zomepirac is an anti-inflammatory drug which was removed from the market due to ADR's. The Zomepirac AG is readily cleaved by β-glucuronidase to give back the parent compound. At pH 7.4, 37^{0}C in an aqueous system, the half life of Zomepirac AG was found to be 27 mins. At physiological pH four other fractions have been isolated other than the Zomepirac AG. Hasegawa *et al.*[13] postulated that the four other isolated compounds were due to acyl migration, as the migratory products are not cleaved by β-glucuronidase.

Telmisartan **(2.20)** (figure 2.3.1) is an angiotensin II receptor antagonist. Telmisartan-acyl glucuronide has been found to be one of the most stable AGs, having a half life of 26h at pH 7.4. This was confirmed by Ebner *et al.*[12] during *in vitro* experiments that indicated very low covalent binding of Telmisartan-acyl glucuronide to human serum albumin (HSA).



(2.19)



(2.20)

Figure 2.3.1: Zomepirac **(2.19)** and Telmisartan **(2.20)**

There is a general trend between the half life of an AG and its reactivity (Table 2.3.2). For drugs such as Tolmetin **(2.21)** and Zomepirac **(2.19)** which have been removed from the market due to ADRs they clearly have shorter half lives compared to Ibuprofen **(1.10)**, which is classed as a relatively safe drug. There are of course other mechanisms which take part in drug toxicity; a measure of AG half life does not give its toxicological profile. Benoxaprofen **(2.3)** has a half life of 2 h, but was withdrawn from the market due to reports of fatal cholestatic jaundice. *(S)*-Naproxen **(2.1)**, which has a half life of 1.8 h is still a drug marketed today and has in the UK become available over the counter as a treatment for primary dysmenorrhoea.

	Half life ($t^{½}$ (h))
Tolmetin **(2.21)**	0.26
Zomepirac **(2.19)**	0.45
Diclofenac **(2.22)**	0.5
(R)-Fenoprofen **(2.23)**	0.98
Indomethacin **(2.24)**	1.4
(R)-Carprofen	1.76
(S)-Fenoprofen	1.93
(S)-Carprofen **(2.25)**	3.09
(S)-Ibuprofen **(1.10)**	3.3
Furosemide **(2.26)**	5.3
Valproic acid **(2.27)**	79

Table 2.3.2: First Order half lifes of 1-*O*-AGs in aqueous buffer, pH 7.4, 37°C[14]

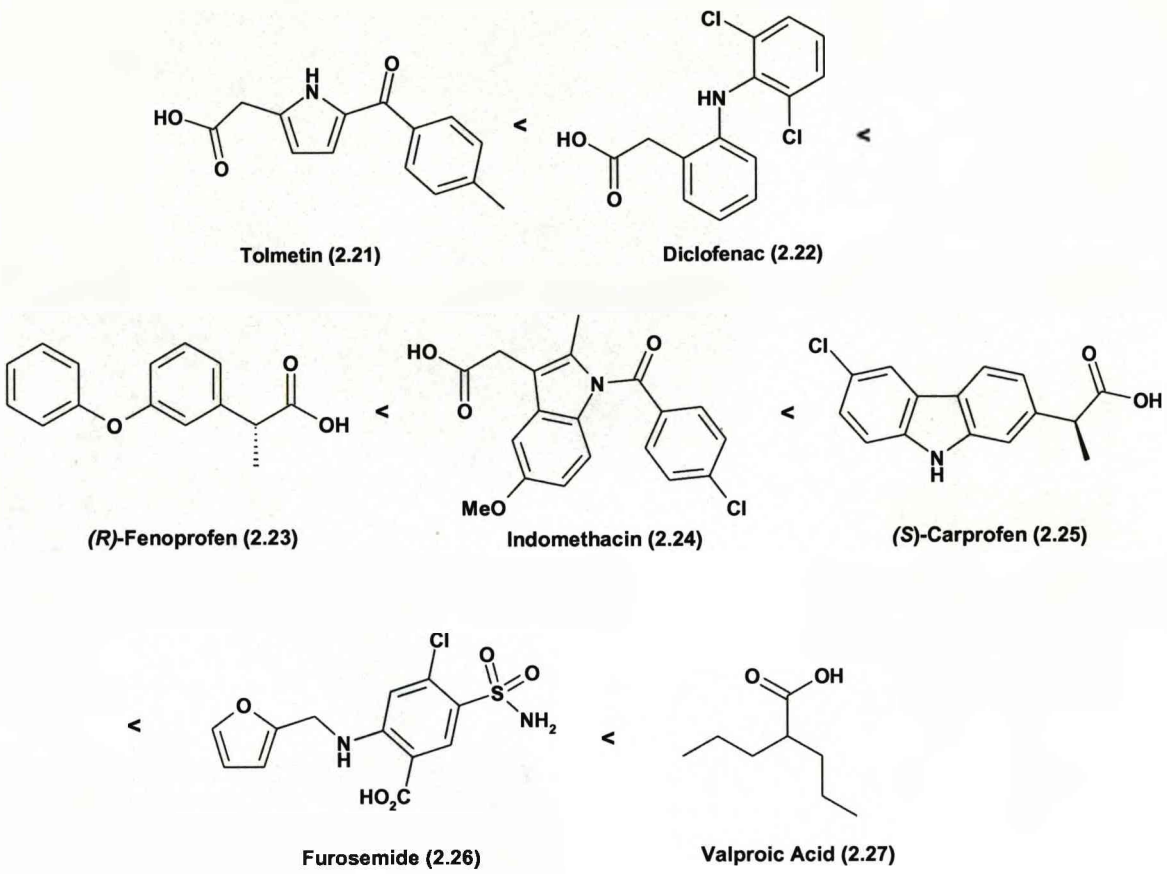


Figure 2.3.3: Compounds that form AGs in order from left to right of shortest half life (see table 2.3.2).

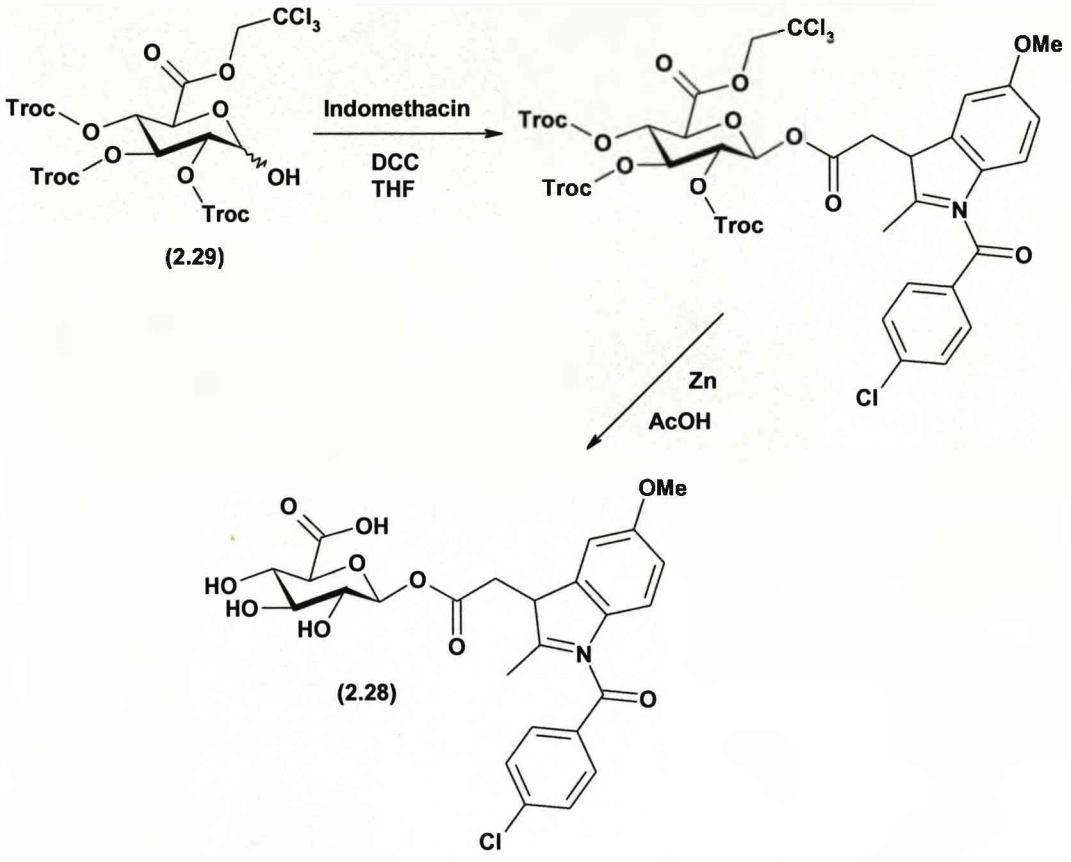
2.4 Previous *Synthesis of 1-β-O-Acyl Glucuronides*

To synthesise *O*-acyl glucuronides in previous years required the use of 'fully' protected sugars[15]. The protecting groups generally used were acetate, benzyl, pivaloate, allyl and silyl. Removal of these groups can be achieved in various ways including hydrolysis, hydrogenation and Pd catalysed removal (table 2.4.1). Such removal of certain protecting groups can be incompatible with the *O*-acyl bond e.g. NaOH hydrolysis of the acetate groups can result in hydrolysis of the *O*-acyl bond.

Protecting Group	Removal
Acetate Pivaloate	1M NaOH/KOH, Or *Aspergillus* lipase[16]
Benzyl	H_2(g), Pd/C
Allyl	$Pd^{(0)}$
Silyl	HF in MeCN

Table 2.4.1: Conditions of removal of well used protecting groups

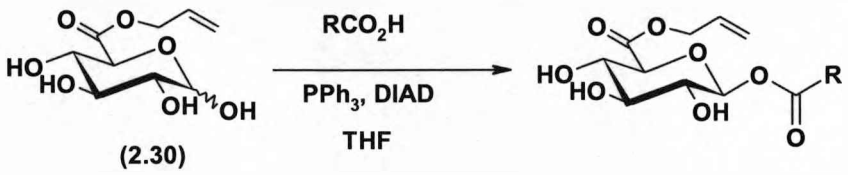
Bugianesi *et al.*[17] synthesised Indomethacin-1β-*O*-acyl glucuronide **(2.28)** from 2,2,2-trichloroethoxycarbonyl (Troc) protected glucuronic acid **(2.29)**. They used DCC in THF to couple Indomethacin in fairly good yields (50 %) (scheme 2.4.2). The synthesis to make the Troc protected sugar was lengthy (six steps) and originated from Tri-*O*-acetyl-1-bromo-α-D-glucopyranuronic methyl ester **(1.6)**. They then removed the Troc protecting groups using Zn dust in acetic acid. This removed the protecting groups successfully but the Zn proved difficult to remove.



Scheme 2.4.2: Conditions for the synthesis of Indomethacin AG **(2.28)**

Protecting groups are frequently utilised in sugar chemistry but it was found recently that when synthesising *O*-acyl glucuronides, selectivity can be obtained at the anomeric OH group to give just the mono-acylated compound. The carboxylic acid moiety of the starting glucuronic acid can be selectively protected which increases the solubility of the glucuronic acid in organic media, but masks the carboxylic acid group during the coupling step. The criteria for the protecting group should be; stability during the coupling step and, ease of removal without disrupting either the *O*-acyl bond or any functional groups within the aglycone.

Juteau *et al.*[18] used allyl glucuronate **(2.30)** as the intermediate in synthesising the acyl glucuronide bond. They used the Mitsunobu method to couple the carboxylic acid to the anomeric position (scheme 2.4.3).



Scheme 2.4.3: Mitsunobu reaction to make the protected *O*-acyl glucuronide

They found that aromatic carboxylic acids gave mixtures of α and β isomers (1:5) during the Mitsunobu coupling with modest yields of 20-40 %. Aliphatic carboxylic acids gave the lowest yields (~20 %) as α/β mixtures.

The Mitsunobu method goes via an S_N2 mechanism giving inversion at the anomeric centre. They were still able to isolate a higher percentage of the β-anomer. This suggests that due to the anomeric effect, the α-phosphonium is the more stable intermediate **(2.31)** (figure 2.4.4). They also observed the formation of the lactone by-product **(2.32)** (figure 2.4.4), which co-eluted with the α-product in a 1:1 ratio.

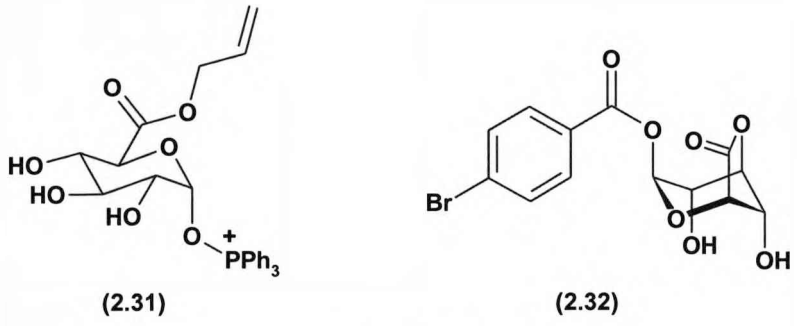
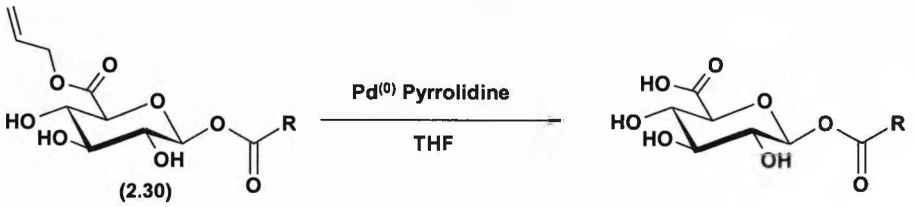


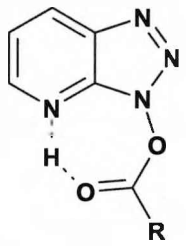
Figure 2.4.4

The last step in the sequence was removal of the allyl protecting group which was accomplished with $Pd^{(0)}$ and pyrrolidine in THF (scheme 2.4.5). Traces of Pd(0) were difficult to remove from the final product.



Scheme 2.4.5: Deprotection of allyl ester glucuronate

Perrie *et al.*[19] found that using HATU to form an activated ester **(2.33)** gave higher yields compared to the Mitsunobu method with >98% β selectivity. They tried HOBt in combination with DIC and found this to be an effective coupling reagent, but HATU gave higher yields. HATU is believed to be superior due to its hydrogen bonding abilities (figure 2.4.6) which renders the carbonyl more electrophilic[20].

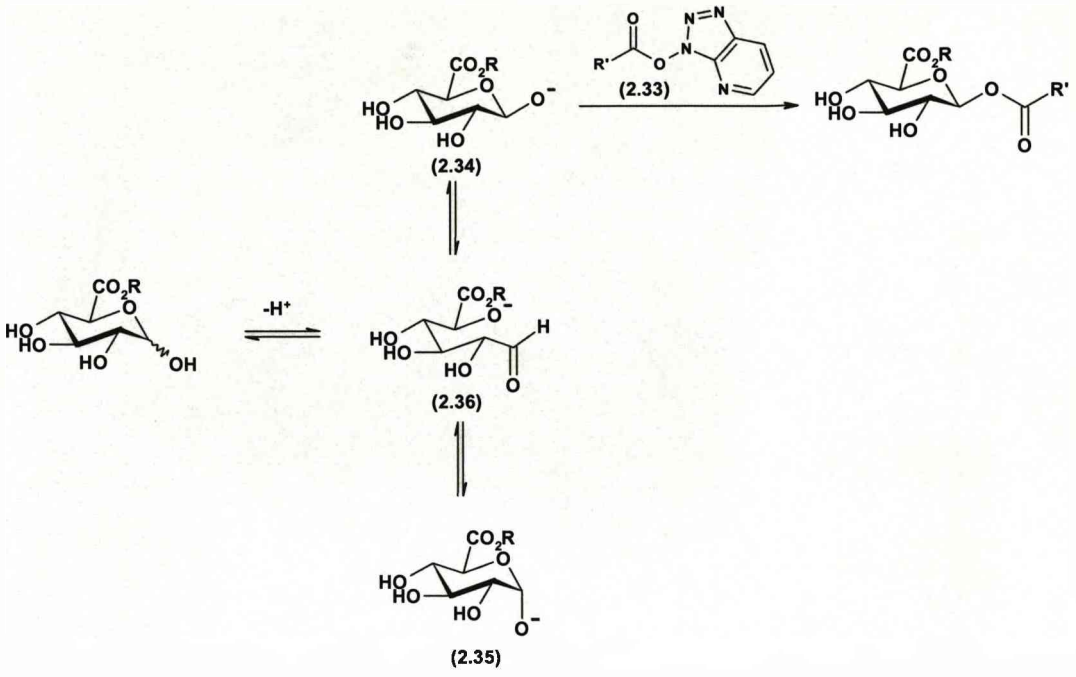


(2.33)

Figure 2.4.6: Hydrogen bonding which can occur with the activated ester of HATU.

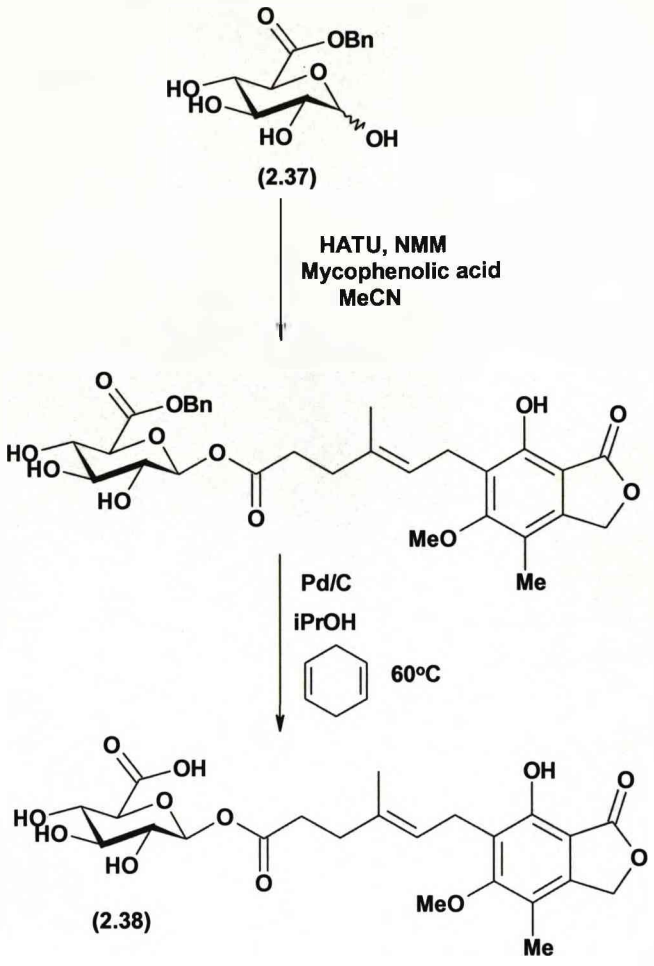
Their studies showed that base strength was critical with N-methyl morpholine (NMM) and DABCO being the favoured bases. They found pyridine was not basic enough and the reaction was slow. Stronger unhindered bases such as 4-dimethylaminopyridine (DMAP) and triethylamine gave overreaction.

The method employed by Perrie *et al.* uses a selective acylation of the 1-*O*-alkoxide. It was found by Schmidt[21,22,23] that the β-alkoxide **(2.34)** is more nucleophilic than the α-alkoxide **(2.35)**. This is suggested to be due to steric effects and the kinetic anomeric effect. The α-alkoxide can often be trapped at lower temperatures owing to its greater thermodynamic stability. An equilibrium between the α and β-alkoxides via the open chain aldehyde **(2.36)** means that the more reactive β-alkoxide reacts with the activated ester **(2.33)** leaving the α-alkoxide to continue to equilibrate (scheme 2.4.7).



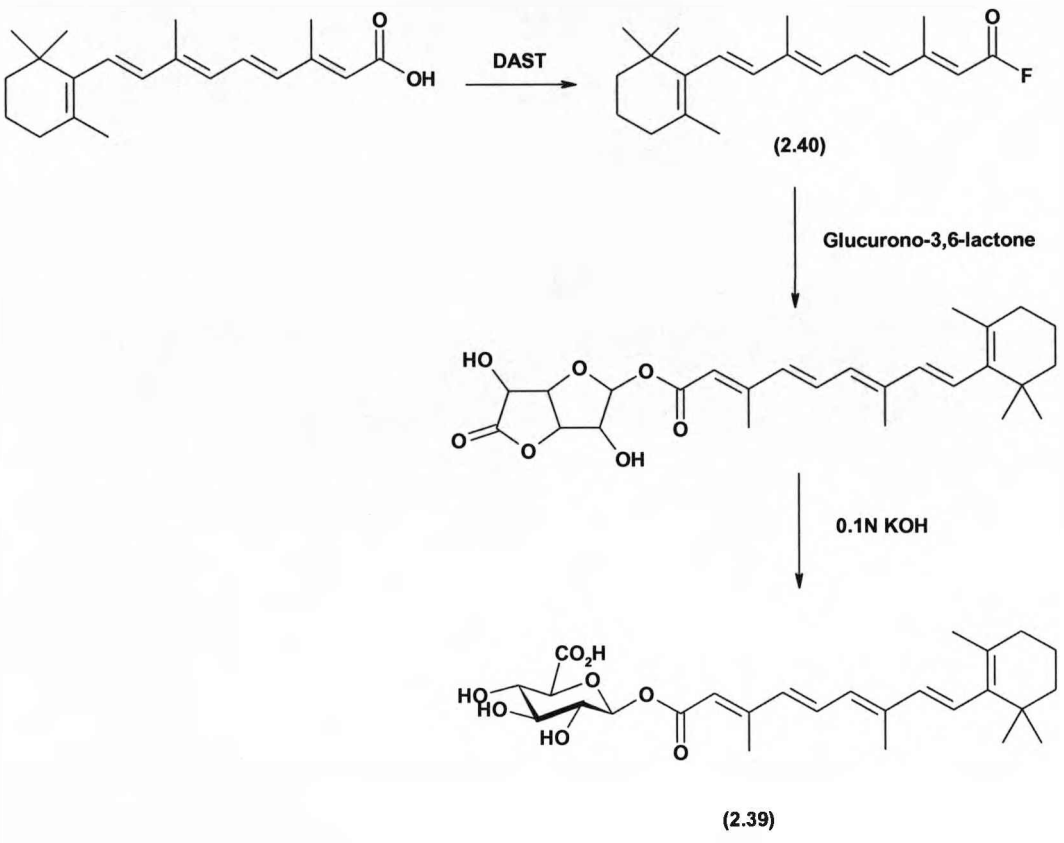
Scheme 2.4.7: The β-alkoxide reacts with the activated ester while the α-alkoxide equilibrates

Bowkett *et al.*[24] focussed their work on the GA protecting group used during the coupling step. They utilised benzyl glucuronate **(2.37)** which is stable during the HATU coupling step, and easily removed by catalytic transfer hydrogenation (or conventional hydrogenation). They were able to remove the benzyl group during hydrogenolysis of mycophenolic AG **(2.38)** without disrupting the double bond present (scheme 2.4.8). Unfortunately, aryl halides were cleaved during the deprotection. They also found formation of isopropyl ester during the deprotection (~2 % by NMR) if the reaction was prolonged. Transesterification could be avoided by using conventional hydrogenation at room temperature.



Scheme 2.4.8: Reaction conditions used by Bowkett to remove the benzyl group

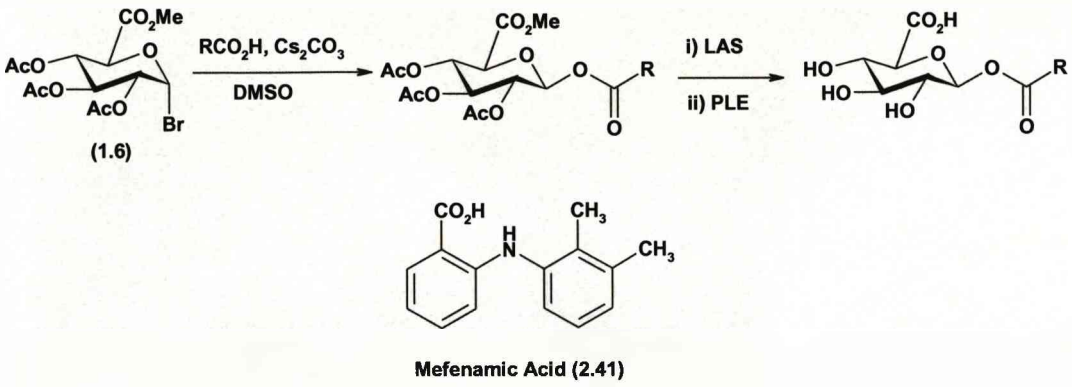
Retinoyl β-glucuronide **(2.39)** was synthesised via the acid fluoride of retinoic acid **(2.40)** (figure 2.4.9) by Barua *et al.*[25]. They synthesised the acid fluoride from the carboxylic acid and DAST. They then reacted the acid fluoride with Glucurono-3,6-lactone which gave mainly the desired product. The coupled glucurono-3,6-lactone was then opened up to give the free acid using 0.1N KOH (scheme 2.4.9).



Scheme 2.4.9

This method works well for retinoic acid but most AGs cannot withstand the alkaline conditions used to open the glucurono-3,6-lactone during the final step.

Baba *et al.*[26] reported the synthesis of AGs of diclofenac **(2.22)**, mefenamic acid **(2.41)** (scheme 2.4.10) and (*S*)-naproxen **(2.1)** using enzymes to selectively remove the acetyl and methyl ester protecting groups. They form the glycosidic bond using an S_N2 reaction between the bromo sugar **(1.6)** and the cesium salt of the drug molecule to give yields of 46-79 %. They then screened various enzymes, but found that lipase AS Amano (LAS) selectively removed the acetyl groups and the methyl ester was removed using esterase from porcine liver (PLE) (scheme 2.4.10).



Mefenamic Acid (2.41)

Scheme 2.4.10

2.5 Aims of the 1-β-O-Acyl Glucuronide project

Previous synthesis of acyl glucuronides has its disadvantages; we therefore believed that further optimisation was required, particularly the protecting group used to mask the glucuronic acid.

The allyl protecting group used by both Juteau and Perrie is removed by Pd (0), but both found Pd residues difficult to remove completely from the final product. It is crucial to remove any heavy metals such as palladium before carrying out *in vivo* and *in vitro* studies, as their presence may affect results. The benzyl glucuronate employed by Bowkett proved to be removed more cleanly than the allyl protecting group, but removal entailed hydrogenation and aromatic halides are not tolerated.

We have also looked at different coupling methods to form the acyl bond. The Mitsunobu method gives mixtures of anomers, low yields, and formation of the lactone by-product mentioned previously (figure 2.4.4). In comparison, using the HATU reagent employed by Perrie gives higher yields and β-selectivity. The disadvantages of using HATU are; the cost of HATU (£40.70 per g), and the inability to perform larger scale reactions (maximum ~0.6 mmol scale).

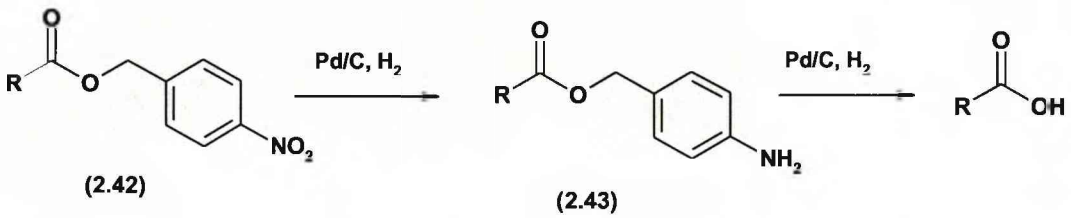
We therefore aimed to find a protecting group where aryl halides would be tolerated during deprotection. We wanted a coupling method which would give higher yields,

be more cost effective and capable of being carried out on larger scales whilst still giving β-selectivity.

2.6 Results and Discussions of the Synthesis of 1β-O-Acyl Glucuronides

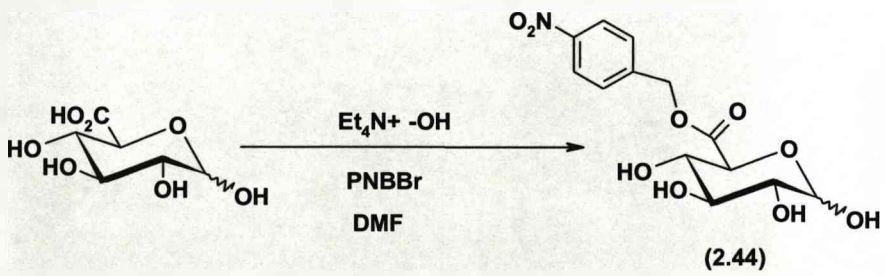
p-Nitrobenzyl Glucuronate

The carboxylic acid moiety of glucuronic acid is a reactive functional group so during the synthesis of acyl glucuronides it requires protecting with a suitable group. The protecting group initially examined was the *p*-nitrobenzyl group (PNB) **(2.42)**. It was thought that the PNB group would be cleaved more readily by hydrogenation than the benzyl ester which is known as a protecting group[24]. The theory was that the nitro group would be reduced first to give *p*-aminobenzyl ester **(2.43)**, which would readily cleave due to the electron donating amino group (scheme 2.6.1).



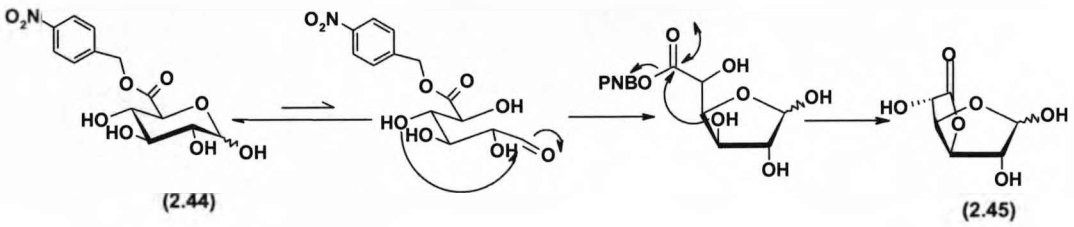
Scheme 2.6.1: Intermediates during the hydrogenation of *p*-nitrobenzyl ester

In the synthesis of the *p*-nitrobenzyl glucuronate **(2.44)** by alkylataion, tetraethylammonium hydroxide was used initially as the base (scheme 2.6.2). This reaction appeared to give many by-products which were difficult to separate from the *p*-nitrobenzyl glucuronate.



Scheme 2.6.2: Synthesis of the PNB glucuronate using tetraethylammonium hydroxide

The product was finally synthesised by using a polymer bound fluoride as base[19,24]. It seems that the base strength is crucial for the success of the reaction, and that the fluoride anion is better than the hydroxyl anion. Another base used for the reaction was tetrabutylammonium fluoride (TBAF), but this appeared to generate a higher yield of glucurono-3,6-lactone. The glucurono-3,6-lactone **(2.45)** is readily formed under basic conditions, with the carbonyl carbon of the ester being attacked by the 3-OH (scheme 2.6.3).



Scheme 2.6.3: Mechanism for the formation of glucurono-3,6-lactone under basic conditions

Deprotection of the PNB group, which is the final step, proved more troublesome than anticipated. The optimisation was carried out on the *p*-chloro substituted benzoic AG **(2.46)**. It was important to retain the chloride during deprotection (figure 2.6.4) to improve upon the benzyl glucuronate deprotection.

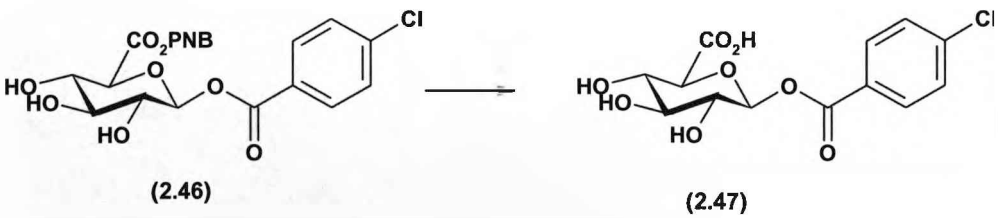


Figure 2.6.4: *p*-chloro substituted benzoic AG used in the optimisation of the deprotection step

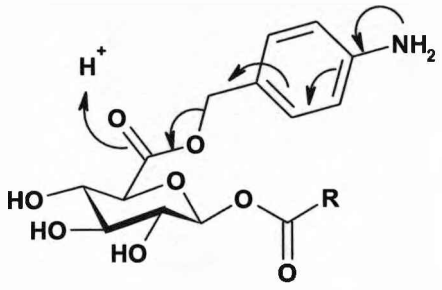
The reaction conditions tried are summarised in table 2.6.5. Rajagopal *et al.*[27] used Palladium catalysed transfer hydrogenolysis with formate salts. Formate salts have hydrogen donating abilities when used in conjunction with Pd/C (10 mol %). We tried

similar conditions to Rajagopal in the hydrogenation of the PNB group (entry 2, table 2.6.5).

	Reaction	***Reagents***	***Solvent***	***Temperature***	***Reaction time***	***Yield***
1	Catalytic transfer hydrogenation	Pd/C, 1,4-cyclohexadiene	iPrOH	60^0C	3h	N.P.I
2	Hydrogenation using Formates	Pd/C, Formic acid, triethylamine	iPrOH	60^0C	4h	N.P.I
3	Catalytic hydrogenation	Pd/C (30 mol %), H_2(g)	iPrOH: THF (2:1)	RT	30mins	60% + Loss of Cl
4	Catalytic hydrogenation	Pd/C (30 mol %), H_2(g), H^+ resin	iPrOH: THF (2:1)	RT	20mins	94%

Table 2.6.5: Reaction conditions tried to deprotect PNB glucuronate **(2.47)**

It was found that when using catalytic hydrogenation (entry 3 in table 2.6.5) aromatic chlorine was replaced by hydrogen. To overcome this problem H^+ resin was added to the reaction mixture (entry 4 in table 2.6.5), which gave the desired product **(2.47)** in 94 % yield. The H^+ resin's role is not entirely clear; thoughts are that it speeds up the removal of the *p*-amino intermediate **(2.48)**, with the reaction going to completion before cleavage of the aryl chloride (figure 2.6.6). The same reaction conditions were tried on the *p*-bromobenzoic AG, but cleavage of the more labile bromine ensued.

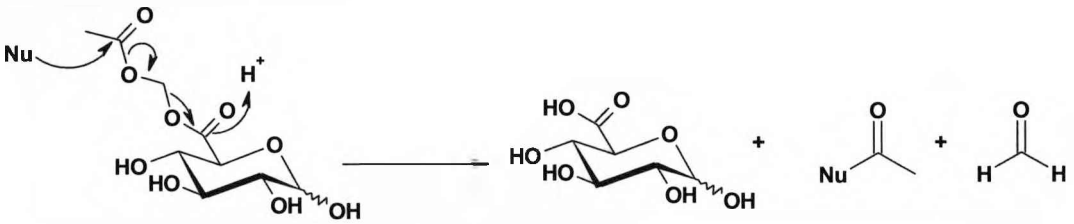


(2.48)

Figure 2.6.6

Acetoxymethyl Protecting Group

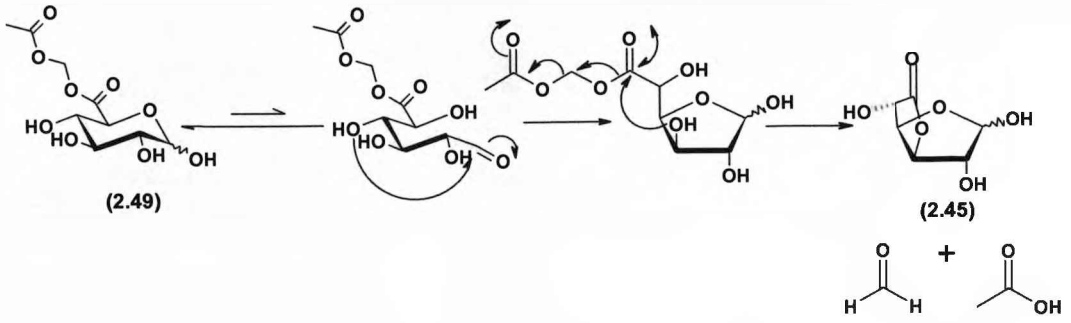
It was thought that a protecting group which could be selectively cleaved using enzyme hydrolysis would be of great use. A 'collapsing' ester glucuronate **(2.49)** (scheme 2.6.7) was initially looked at and the hopes were that it would be easily removed by either enzyme or very mild chemical hydrolysis. Such groups are used in prodrugs, which are then metabolised *in vivo* to the active form of the drug.



(2.49)

Scheme 2.6.7: Mechanism for the removal of the 'collapsing' ester group

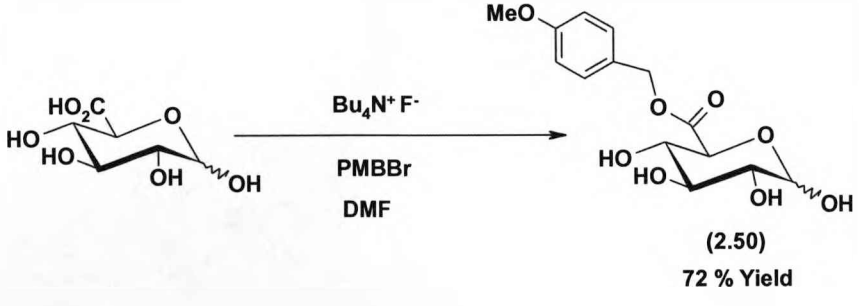
To introduce this protecting group several methods were investigated. Unfortunately on analysis of the reaction it was observed that the major product was in fact the glucurono-3,6-lactone **(2.45)**. This is formed readily due to the labile nature of the protecting group, which can also act as a leaving group (scheme 2.6.8).



Scheme 2.6.8: Mechanism to form the glucurono-3,6-lactone from the 'collapsing' ester

***p*-Methoxybenzyl Glucuronate**

Another protecting group that we looked at was *p*-methoxybenzyl (PMB). The same conditions used to synthesise the *p*-nitrobenzyl glucuronate (polymer bound fluoride) were used to synthesise *p*-methoxybenzyl glucuronate **(2.50)**. Tetrabutylammonium fluoride (TBAF) (scheme 2.6.9) can be used as an alternative base for this reaction, as the formation of the glucuronolactone **(2.45)** is less favourable. This is probably due to the donating effect of the *p*-methoxy group.



Scheme 2.6.9: Reaction conditions when using TBAF as base

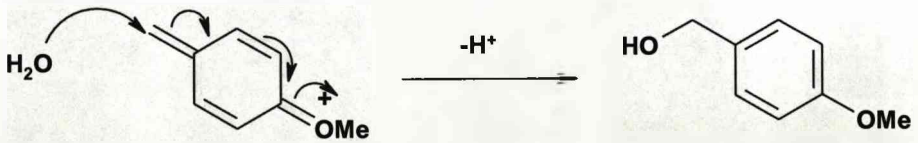
It was thought that the *p*-methoxybenzyl group would be easily removed by mild acid hydrolysis. Various conditions were tried: these are summarised in table 2.6.10. The conditions that gave optimal results were 10 % v/v solution of TFA (90 % aq) in DCM (0.1g/ml).

	Reaction	*Reagents*	*Solvent*	*Temp*	*Reaction time*	*Yield*
1	Mild acid hydrolysis	Formic acid (XS)	Dioxane	60^{0}C	5h	no reaction observed
2	Catalytic transfer hydrogenation	Pd/C(20mol%) 1,4-cyclohexadiene	Dioxane	60^{0}C	5h	N.P.I
3	Mild acid hydrolysis	Amberlite H^+ resin	Dioxane	60^{0}C	~24h	no reaction observed
4	Mild acid hydrolysis	TFA (90 % aq) (10%v/v)	DCM	0^{0}C to RT	2-5h	52-98%

Table 2.6.10: Summary of conditions used to remove the *p*-methoxybenzyl protecting group

In retrospect it may have been the use of the polar solvent dioxane which may have inhibited the hydrolysis of the PMB group; it is potentially a good Lewis base.

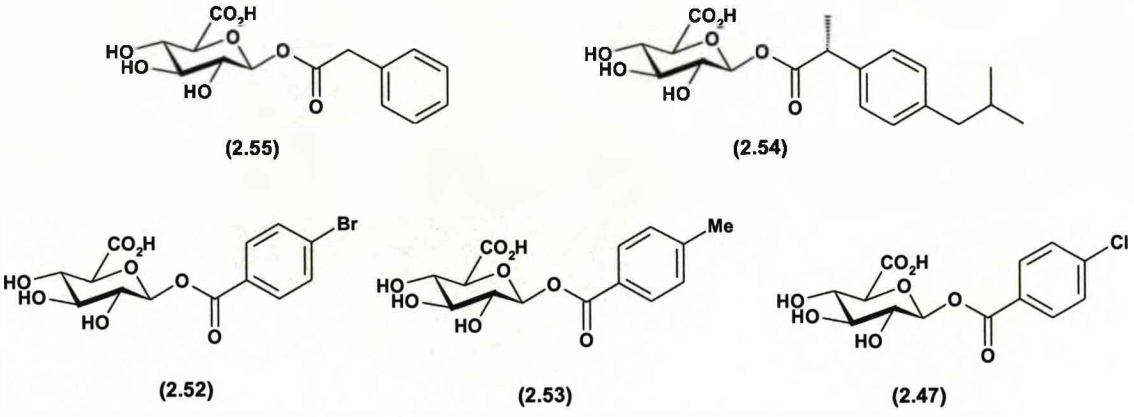
It was also important to have water present during the deprotection to react with the cation generated **(2.51)** (scheme 2.6.11), without the presence of water no reaction was observed.



(2.51)

Scheme 2.6.11: Water as a cation scavenger in the PMB deprotection reaction

The AGs formed using using this method of deprotection are summarised in figure 2.6.12.

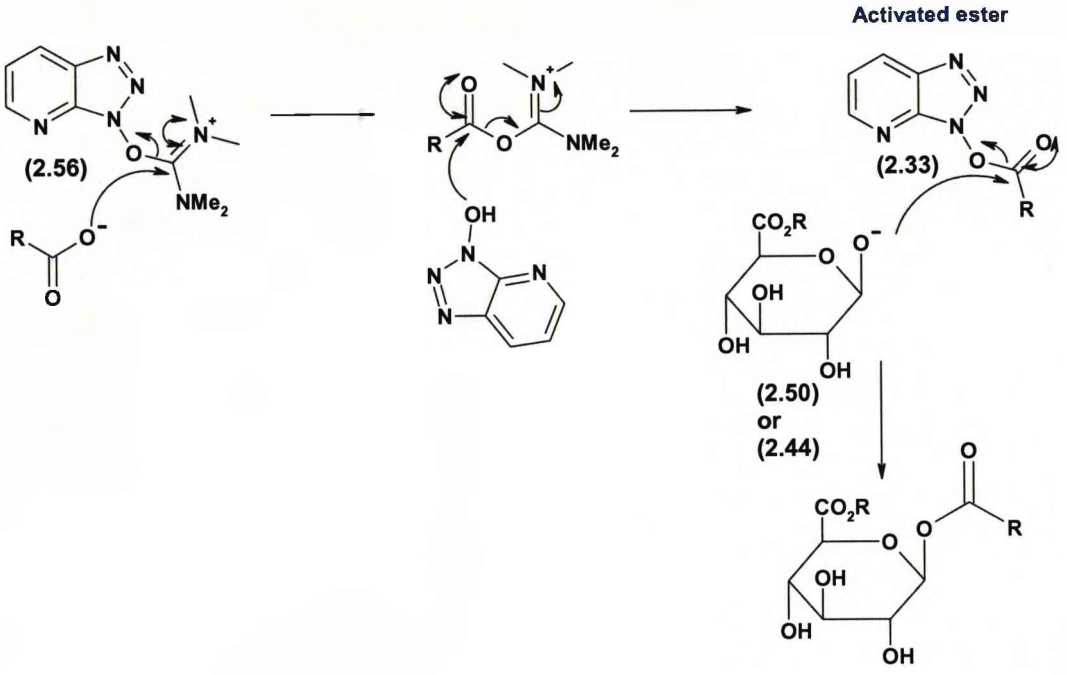


Scheme 2.6.12: Acyl glucuronides synthesised by the PMB protected GA, via HATU coupling (see next pg).

Coupling Methods

Peptide Coupling Reagents

The reagent we initially chose to use was HATU **(2.56)**; we used the same conditions as Perrie *et al.*[19] to exploit the effectiveness of our PMB Glucuronate **(2.50)** and PNB Glucuronate **(2.44)** in the reaction (scheme 2.6.13).



Scheme 2.6.13: Mechanism for the coupling step using HATU to activate the carboxylic acid

The coupling method using HATU was carried out with various carboxylic acids comprising, primary, secondary, and substituted benzoic acids to give the protected AGs summarised in figure 2.6.14.

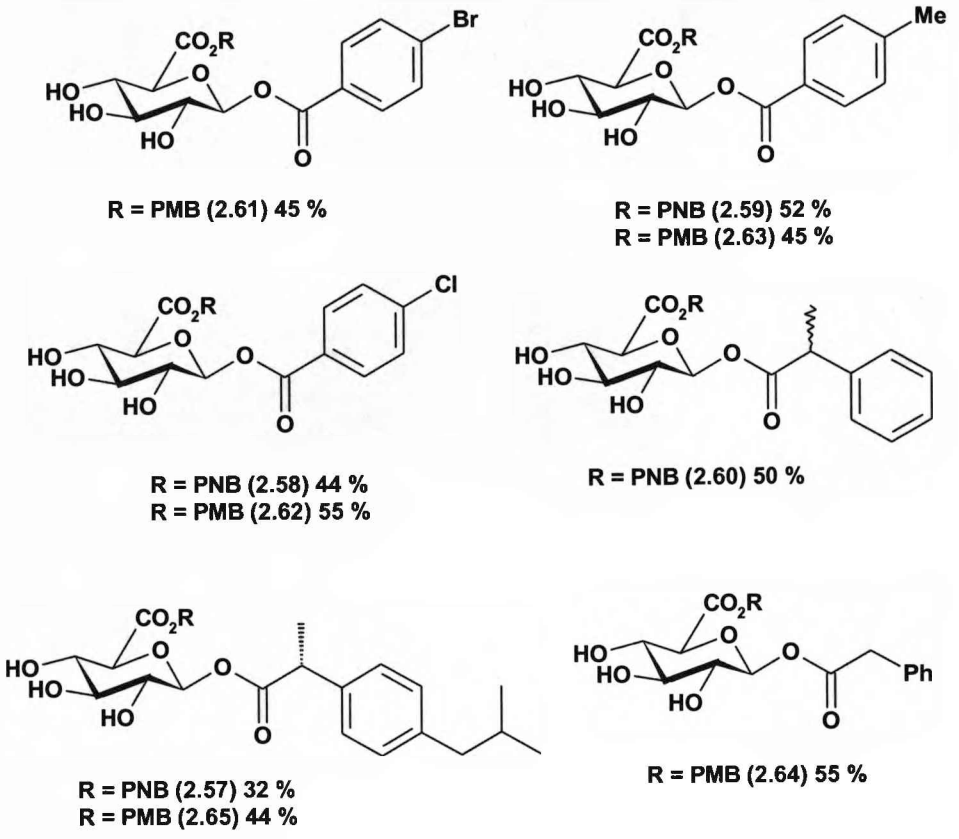


Figure 2.6.14: Products formed using the HATU method

We found that the PMB glucuronate **(2.50)** and PNB glucuronate **(2.44)** gave yields comparable to the benzyl **(2.37)** and allyl glucuronate **(2.3)** during the coupling reaction using HATU. We therefore moved on to look at other coupling methods as we had initially intended.

Other coupling reagents have been looked at: these included HOBt **(2.66)**, and PFP **(2.67)** with either DIC **(2.68)** or DCC **(2.69)** (figure 2.6.15).

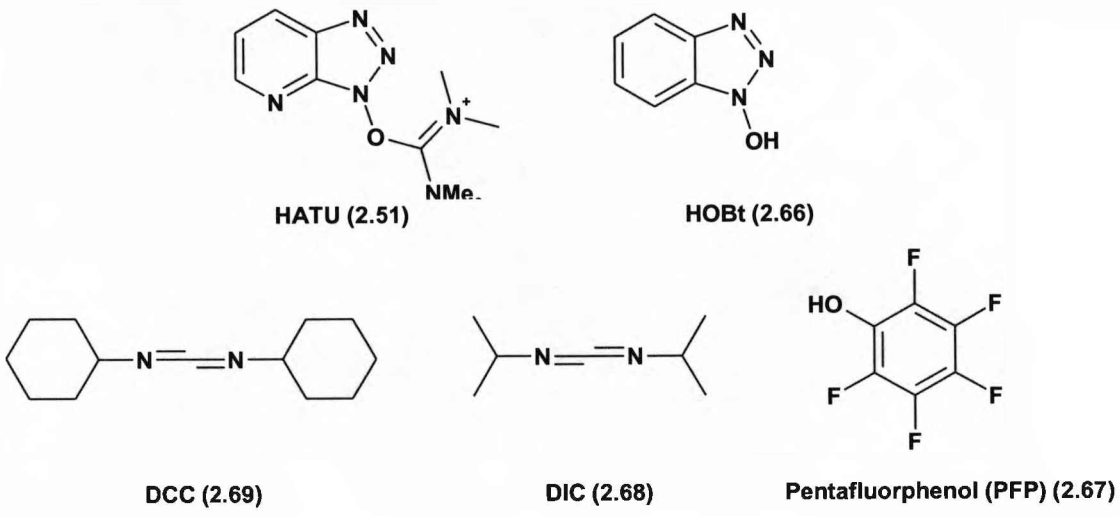
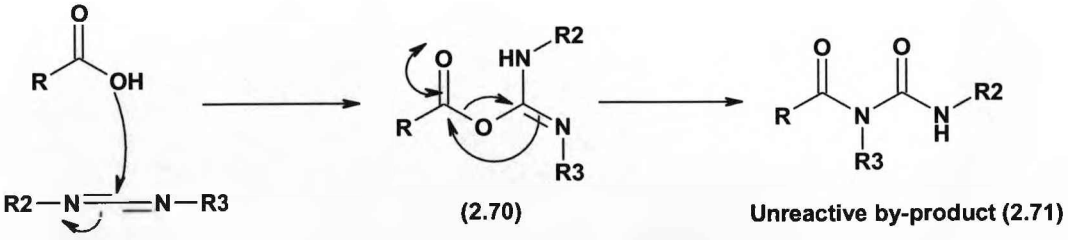


Figure 2.6.15: Coupling reagents

DIC **(2.68)** is thought to react with the carboxylic acid initially; this forms an *O*-acyl urea **(2.70)** (scheme 2.6.16) that undergoes reaction with HOBt **(2.66).** We found that HOBt with DIC was less effective than HATU **(2.51)**, giving yields of 26 % with phenyl acetic acid (HATU gives 55 % with phenyl acetic acid). DIC used on its own can cause problems as once the *O*-acyl isourea is formed it can then react with itself to form an *N*-acylurea **(2.71)** (scheme 2.6.15).



Scheme 2.6.16: Formation of the unreactive N-acylurea from DCC or DIC type reagents

Another reaction carried out was to use a combination of HOBt **(2.66)**, DIC **(2.68)** and pentafluorophenol (PFP) **(2.67)**. With the addition of the PFP, the final activated derivative is understood to be the PFP ester **(2.72)** (figure 2.6.17). This is a better activating group in comparison to HOBt **(2.73)** due to the electron withdrawing nature of the fluorines, which enhance the electrophilic nature of the carbonyl.

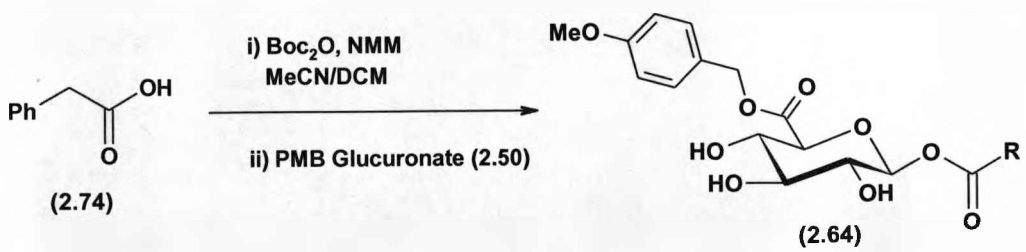
(2.72) (2.73)

Figure 2.6.17: Activated ester of PFP **(2.72)** and HOBt **(2.73)**

This reaction gave a 26 % yield which was less efficient than the HATU method which gives 40-70 % yields. To study this further an experiment was performed with DIC **(2.68)** and PFP **(2.67)**, i.e. removal of HOBt. There is literature evidence of successful peptide coupling reactions using PFP and DCC[28]. No product was isolated from this reaction suggesting that the previous result was from formation of the HOBt activated ester **(2.73)** (figure 2.6.17).

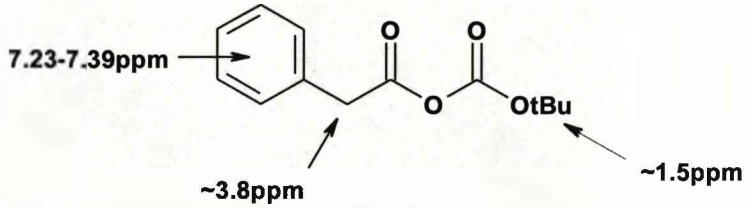
Mixed Anhydrides

Other ways of activating carboxylic acids include forming; mixed anhydride, acid chlorides and acid fluorides. Mixed anhydrides have been used successfully in peptide coupling chemistry[29]. We first looked at using di-tert-butyl dicarbonate (Boc anhydride [Boc_2O]) and phenyl acetic acid to form the required mixed anhydride. The phenyl acetic acid **(2.74)**, Boc_2O and NMM were reacted together initially for 1h before adding the PMB glucuronate **(2.50)** (scheme 2.6.18).



Scheme 2.6.18: Reaction conditions for the mixed anhydride method

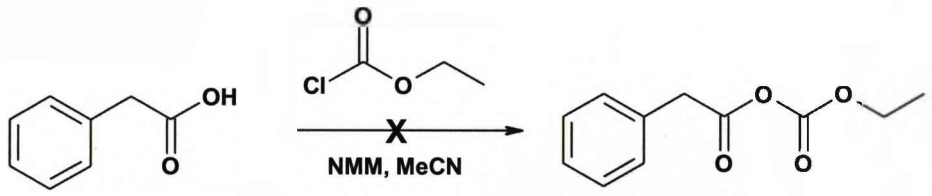
On analysis of this reaction it appeared that the major component of the reaction was the mixed anhydride **(2.75)**, suggesting that the mixed anhydride was not reactive enough (figure 2.6.19). Only a 6 % yield of the desired β-product **(2.64)** was isolated, no α-anomer was isolated.



(2.75)

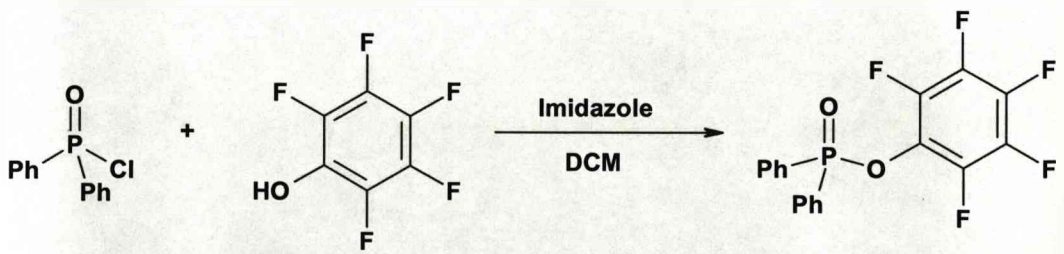
Figure 2.6.19: The mixed anhydride of phenyl acetic acid and the NMR chemical shifts

It was thought that the *t*-butyl group may be too sterically hindered reducing the reactivity. DABCO (pKa 8.8), which is a stronger base, was tried but this gave no product. The next reagent investigated to form the mixed anhydride was ethylchloroformate; this gave no reaction at all (scheme 2.6.20).



Scheme 2.6.20

Pentafluorophenol diphenylphosphinate (FDDP) **(2.76)** mixed anhydrides[30] were studied next. The intermediate FDDP **(2.76)** was synthesised from diphenylphosphinic chloride and pentafluorophenol (scheme 2.6.21).



(2.67) **(2.76)**

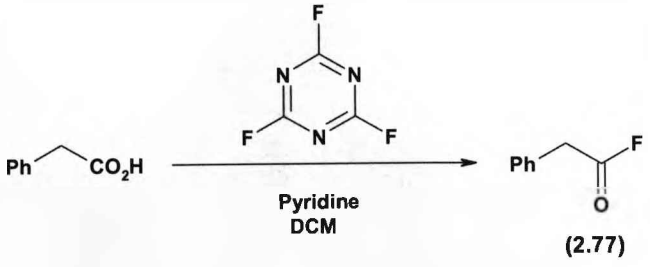
Scheme 2.6.21: Reaction conditions for the synthesis of FDDP

Once the FDDP **(2.76)** had been isolated it was reacted on with phenyl acetic acid for 30mins before addition of the PMB glucuronate **(2.50)**. By TLC there appeared to be some desired product, but many by-products. On purification of the reaction it was difficult to separate the desired product from by-products. The crude yield after purification was 44 % (with purity of ~60 %); it was decided that this route was not worth pursuing.

Acid Fluorides

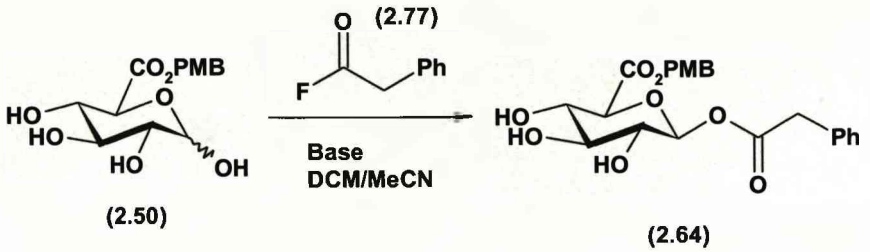
Acid fluorides have been used in peptide coupling chemistry by Carpino *et al*[31] when dealing with acid sensitive protected amino acids. They noted that acid fluorides have a greater stability compared to acid chlorides towards neutral oxygen nucleophiles such as water or methanol, but appear to have nearly equal reactivity toward anionic nucleophiles.

To synthesise the acid fluoride we used cyanuric fluoride and pyridine (scheme 2.6.22), similar to the method of Kokotos and Noula[32]. The reaction went in quantitative yield and was analysed by IR; the acid fluoride carbonyl stretch was measured at 1844.8 cm^{-1} compared to the carboxylic acid stretch which is 1709.6 cm^{-1}.



Scheme 2.6.22: Reaction conditions for the formation of phenylacetyl fluoride

The acid fluoride **(2.77)** was added to the coupling reaction with PMB Glucuronate **(2.50)** as a solution in DCM (scheme 2.6.23). Several reactions were carried out under various conditions (table 2.6.24).



(2.50) (2.64)

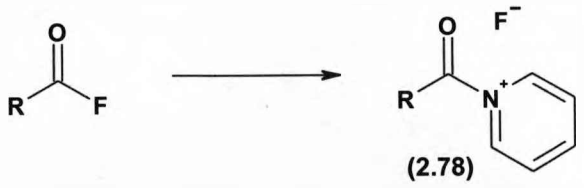
Scheme 2.6.23: Coupling reaction of PMB Glucuronate and phenylacetyl fluoride

	Base (eq)	***Solvent***	***Reaction Time***	***Reaction Temp***	***Yield***
1	NMM (2eq)	MeCN/DCM	5h	RT	31% and diacylated product
2	DABCO (2eq)	MeCN/DCM	5h	RT	N.P.I
3	NMM (1eq)	MeCN/DCM	5h	RT	27% and diacylated product
4	Pyridine (1eq)	MeCN/DCM	5h	RT	diacylated product only
5	NMM(1eq)	MeCN/DCM	6.5h	0^0C	40%
6	NMM (1eq)	MeCN/DCM	6.5h	-10^0C	36%

Table 2.6.24: Reaction Conditions used with phenyl acetyl fluoride and PMB glucuronate

We started with two equivalents of NMM, but found that there was over reaction giving diacylation. We then reduced the NMM to one equivalent and found that the diacylation was not reduced to any extent. Reducing the temperature from RT to 0^0C increased the yield from 27 % to 40 % (Entries 3 & 5 in table 2.6.24), and reduced the diacylated product. Using DABCO as a base resulted in no desired product (Entry 2 in table 2.6.24). Pyridine gave only the diacylated product (Entry 4 in table 2.6.24). It is

possible that the pyridinium salt is formed which results in a more reactive pyridinium species **(2.78)** (scheme 2.6.25).



(2.78)

Scheme 2.6.25: Formation of the more reactive pyridinium species

2.7 Conclusions and future work

Due to the synthesis of AGs we have been able to investigate SAR between increased α-substituted phenyl acetic acid and k_d and have studied the differences in *(R)* and *(S)*-ibuprofen with respect to acyl migration. This has given more insight into the relationship between structure and k_d. The pharmaceutical industry is becoming more concerned about toxic metabolites so it is important to investigate such relationships.

We have found the use of PMB glucuronate a good alternative to benzyl glucuronate when trying to synthesise AGs of carboxylic acids containing aromatic halogens. We also avoid the use of heavy metals during deprotection meaning the compounds made can be used in proteomic and *in vivo* studies.

The acyl fluoride chemistry requires further optimisation, but could be used as an alternative to HATU. Initially we set out to find a cheaper, higher yielding and scalable alternative. Although the yields have not superseded those of HATU, the reagents used to synthesise the acid fluoride are cheaper than HATU (cyanuric fluoride £8.50 per g, HATU £40.70 per g). The reactions also appear to tolerate solvents other than acetonitrile; the HATU method does not tolerate other solvents. We were able to carry out the reaction on a 2 mmol scale, giving similar yields to the respective 0.6 mmol reaction. This is not possible when using HATU; using greater than 0.6 mmol of glucuronate gives lower yields of product.

2.8 References

(1) Ikegawa, S.; Murao, N.; Oohashi, J.; Goto, J. *Biomedical Chromatography* **1998**, *12*, 317-321.

(2) Bailey, M. J.; Dickinson, R. G. *Chemico-Biological Interactions* **2003**, *145*, 117-137.

(3) SpahnLangguth, H.; Dahms, M.; Hermening, A. In *5th International Symposium on Biological Reactive Intermediates*; Snyder, R. K. J. J. S. I. G. K. G. F. J. D. J. G. H. M. T. J. W. C. M., Ed. Munich, Germany, 1995, p 313-328.

(4) Shipkova, M.; Armstrong, V. W.; Oellerich, M.; Wieland, E. *Therapeutic Drug Monitoring* **2003**, *25*, 1-16.

(5) Corcoran, O.; Mortensen, R. W.; Hansen, S. H.; Troke, J.; Nicholson, J. K. *Chemical Research in Toxicology* **2001**, *14*, 1363-1370.

(6) Vanderhoeven, S. J.; Troke, J.; Tranter, G. E.; Wilson, I. D.; Nicholson, J. K.; Lindon, J. C. *Xenobiotica* **2004**, *34*, 889-900.

(7) Johnson, C. H.; Wilson, I. D.; Harding, J. R.; Stachulski, A. V.; Iddon, L.; Nicholson, J. K.; Lindon, J. C. *Analytical Chemistry* **2007**, *79*, 8720-8727.

(8) Skordi, E.; Wilson, I. D.; Lindon, J. C.; Nicholson, J. K. *Xenobiotica* **2005**, *35*, 715-725.

(9) Hasegawa, H.; Akira, K.; Shinohara, Y.; Kasuya, Y.; Hashimoto, T. *Biological & Pharmaceutical Bulletin* **2001**, *24*, 852-855.

(10) Berry, N. Iddon, L. *Organic and Biomolecular Chemistry* **2009**, *7*, 2525-2533.

(11) Bischer, A.; ZiaAmirhosseini, P.; Iwaki, M.; McDonagh, A. F.; Benet, L. Z. *Journal of Pharmacokinetics and Biopharmaceutics* **1995**, *23*, 379-395.

(12) Ebner, T.; Heinzel, G.; Prox, A.; Beschke, K.; Wachsmuth, H. *Drug Metabolism and Disposition* **1999**, *27*, 1143-1149.

(13) Hasegawa, J.; Smith, P. C.; Benet, L. Z. *Drug Metabolism and Disposition* **1982**, *10*, 469-473.

(14) Stachulski, A. V.; Harding, J. R.; Lindon, J. C.; Maggs, J. L.; Park, B. K.; Wilson, I. D. *Journal of Medicinal Chemistry* **2006**, *49*, 6931-6945.

(15) Smith, A. B.; Hale, K. J.; Rivero, R. A. *Tetrahedron Letters* **1986**, *27*, 5813-5816.

(16) Stachulski, A. V.; Jenkins, G. N. *Natural Product Reports* **1998**, *15*, 173-186.

(17) Bugianes.R; Shen, T. Y. *Carbohydrate Research* **1971**, *19*, 179-&.

(18) Juteau, H.; Gareau, Y.; Labelle, M. *Tetrahedron Letters* **1997**, *38*, 1481-1484.

(19) Perrie, J. A.; Harding, J. R.; Holt, D. W.; Johnston, A.; Meath, P.; Stachulski, A. V. *Organic Letters* **2005**, *7*, 2591-2594.

(20) Carpino, L. A. *Journal of the American Chemical Society* **1993**, *115*, 4397-4398.

(21) Schmidt, R. R.; Michel, J. *Tetrahedron Letters* **1984**, *25*, 821-824.

(22) Cinget, F.; Schmidt, R. R. *Synlett* **1993**, 168-170.

(23) Tsvetkov, Y. E.; Klotz, W.; Schmidt, R. R. *Liebigs Annalen Der Chemie* **1992**, 371-375.

(24) Bowkett, E. R.; Harding, J. R.; Maggs, J. L.; Park, B. K.; Perrie, J. A.; Stachulski, A. V. *Tetrahedron* **2007**, *63*, 7596-7605.

(25) Barua, A. B.; Olson, J. A. *Journal of Lipid Research* **1985**, *26*, 1277-1282.

(26) Baba, A.; Yoshioka, T. *Organic & Biomolecular Chemistry* **2006**, *4*, 3303-3310.

(27) Rajagopal, S.; Spatola, A. F. *Journal of Organic Chemistry* **1995**, *60*, 1347-1355.

(28) Kovacs, J.; Kisfalud.L; Ceprini, M. Q. *Journal of the American Chemical Society* **1967**, *89*, 183

(29) Chen, F. M. F.; Lee, Y.; Steinauer, R.; Benoiton, N. L. *Canadian Journal of Chemistry-Revue Canadienne De Chimie* **1987**, *65*, 613-618.

(30) Chen, S. Q.; Xu, J. C. *Tetrahedron Letters* **1991**, *32*, 6711-6714.

(31) Carpino, L. A.; Sadataalaee, D.; Chao, H. G.; Deselms, R. H. *Journal of the American Chemical Society* **1990**, *112*, 9651-9652.

(32) Kokotos, G.; Noula, C. *Journal of Organic Chemistry* **1996**, *61*, 6994-6996.

Chapter Three
1-β-O-Acyl glucosides

3.1 Introduction to 1-β-O-Acyl Glucosides

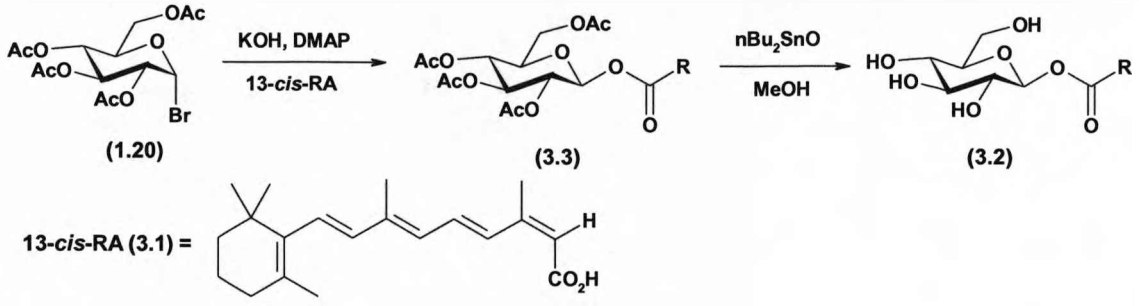
In mammals glucuronidation is known as the main metabolic pathway for xenobiotics and endogenous compounds. In plants, bacteria, insects and molluscs glycosylation has been well documented[1-3] as a significant metabolic pathway.

Plants use glycosylation to detoxify xenobiotic and endogenous compounds such as 2,4,5-trichlorophenol. The detoxification of compounds can be broken down into three stages. The first stage is transformation (phase I), followed by conjugation (phase II), and then internal compartmentalisation and storage (phase III), with storage sites being the vacuole or cell wall.[4,5]

More examples of glycosylation as a detoxification process and where it occurs have already been mentioned, and can be found in the general introduction.

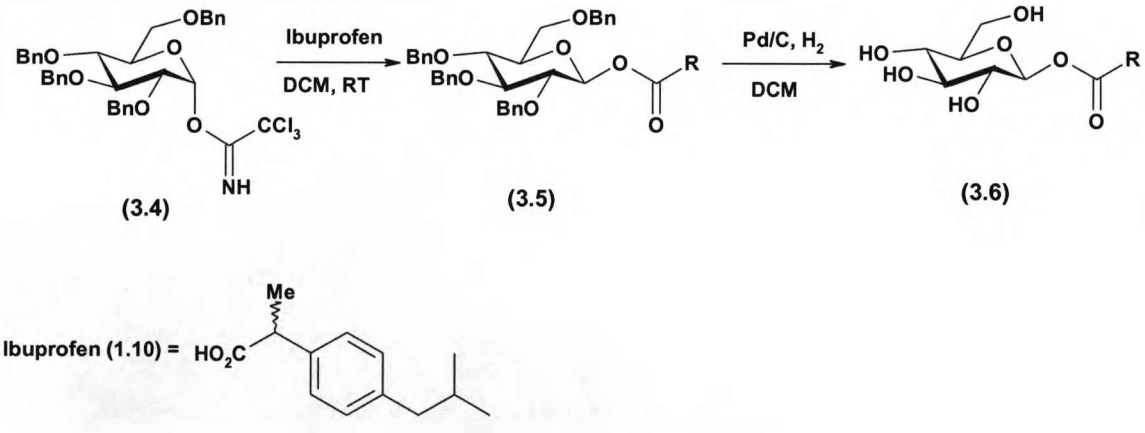
3.2 Previous Synthesis of 1-β-O-Acyl Glucosides

Retinoic acids such as *all*-trans-retinoic acid (ATRA), 13-*cis*-retinoic acid (13-*cis*-RA) **(3.1)** (scheme 3.2.1), and other retinoids are currently used for treatment of dermatological disorders and chemotherapeutic agents against various endothelial cancers, breast cancer, and endometrial cancer. These compounds are too toxic in higher mammals for the prevention of cancer, and many side effects have limited their use. Retinoid Acyl glucosides **(3.2)** have been synthesised in an attempt to form less toxic compounds for the treatment of cancer. Xiang *et al.*[6] took the bromo-sugar **(1.20)** and 13-*cis*-RA **(3.1)** in the presence of KOH with DMAP as catalyst to give the product in 53 % yield (scheme 3.2.1). They then deprotected **(3.3)** using dibutyltin oxide as a catalyst in methanol to give the final product in 50 % yield (scheme 3.2.1).



Scheme 3.2.1

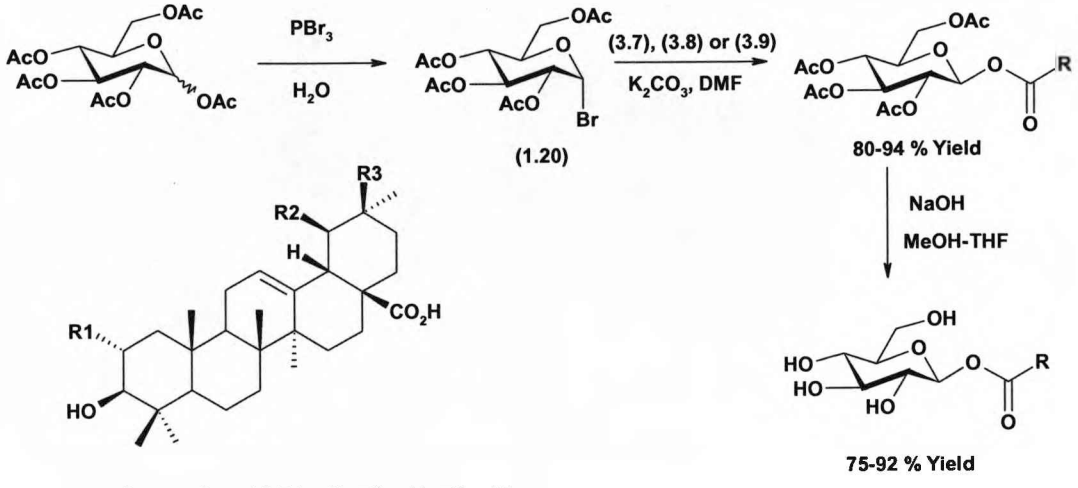
There are examples of acyl glucoside conjugates of drugs such as Ibuprofen **(1.10)**. Uhrig *et al.*[7] hoped that by adding the glucose moiety, they would increase the bioavailabilty of ibuprofen. They used the trichloroacetimidate sugar **(3.4)** to form the conjugated intermediate, which gave them the desired product **(3.5)** in 67 % yield. Deprotection of the benzyl groups was carried out by hydrogenation giving a 76 % yield of **(3.6)** (scheme 3.2.2). They found the product **(3.6)** to be very unstable, even when stored at -20°C.



Scheme 3.2.2

Wen *et al.*[8] synthesised three pentacyclic triterpene glycoconjugates to evaluate structure activity relationships for the inhibition of rabbit muscle glycogen phosphorylase a (GPa). They found that these compounds had reduced activity. To synthesise the conjugates they first formed the bromo sugar **(1.20)** and then

coupled this to the triterpenes; oleanolic acid **(3.7)**, ursolic acid **(3.8)** and corosolic acid **(3.9)**, in the presence of potassium carbonate. The intermediates were then deprotected using NaOH in methanol and THF (scheme 3.2.3).



(3.7) Oleanolic acid R1 = H, R2 = H, R3 = Me
(3.8) Ursolic acid R1 = H, R2 = Me, R3 = H
(3.9) Corosolic acid R1 = OH, R2 = Me, R3 = H

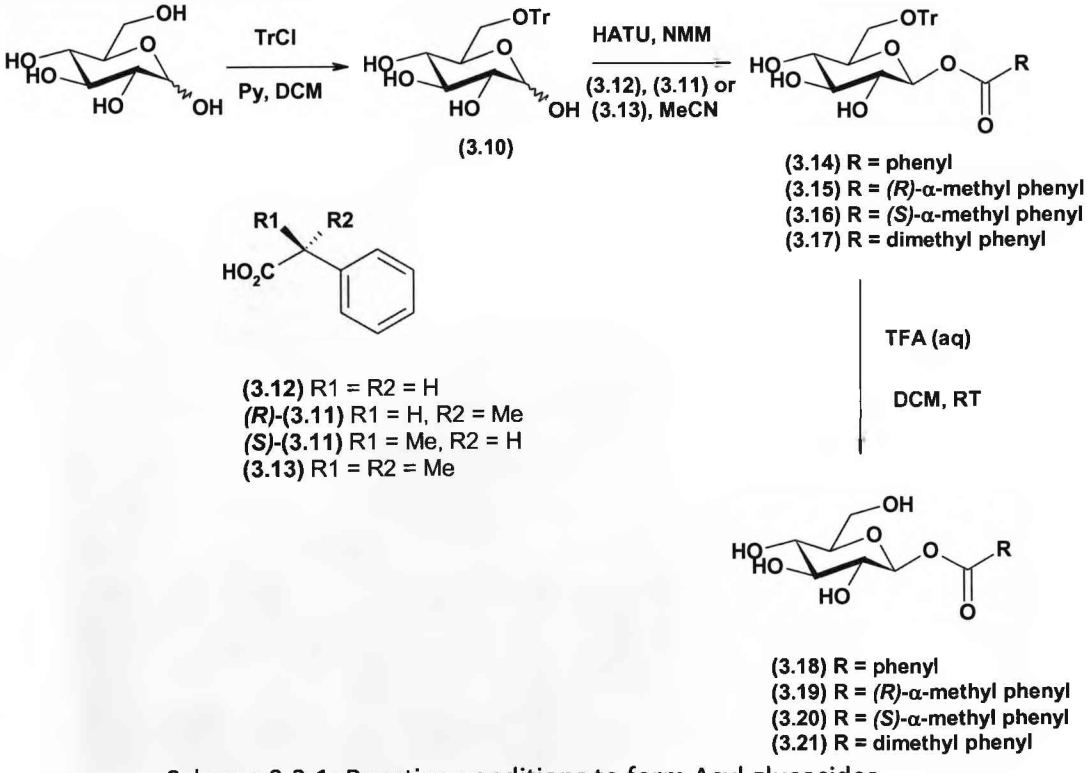
Scheme 3.2.3

3.3 Results and Discussions of the Synthesis of 1-β-O-Acyl Glucosides

Our aim in synthesising 1-β-*O*-acyl glucosides was to study acyl migration by NMR in the glucose series. We chose the phenyl acetic acid series, with increasing α-methyl substitution so that we could compare our results to the glucuronic acid series.

Synthesis

We decided to use 6-*O*-trityl glucose **(3.10)** as our glucose source; we knew that this was easily made, and is a stable intermediate. To form the glycosidic bond we used our selective acylation method, using HATU to form the activated ester, which gave us the intermediates in 49-55 % yield (scheme 3.3.1). At this stage the diastereoisomers of the mono-methyl derivatives **(3.11)** were separated by chromatography. The deprotection step was then carried out using TFA (aq) in DCM, and was complete within 30 minutes as determined by TLC (scheme 3.3.1). The products were purified by column chromatography, to give yields of 88-92 %.



Scheme 3.3.1: Reaction conditions to form Acyl glucosides

The compounds were then studied by NMR at Imperial College London by Caroline Johnson. Each compound was dissolved in a buffer solution of sodium phosphate (pH 7.4). The degradation rate (k_d) was then measured by taking the integration of the 1-β-anomeric proton at the beginning of the reaction and at various time points. A plot of ln (anomeric proton integral at timepoint/anomeric proton integral at t = 0) vs timepoint gives k_d (the slope of the graph). This gives the k_d value for migration from the 1-β-position to the 2-position i.e. the initial migration (scheme 2.2.1, pg. 36). Half life is calculated from k_d using the equation: $t_{1/2} = \ln 2/k_d$

The half lives measured for the four acyl glucosides are shown in table 3.3.2, and those measured for the equivalent glucuronides are shown in table 3.3.3 for comparison.

Conjugated acid to glucose	***k_d (h^{-1})***	***$t_{1/2}$ (h)***
phenylacetic acid **(3.12)**	1.419	0.49
R-α-methyl phenylacetic acid **(3.11)**	0.782	0.89
S-α-methyl phenylacetic acid **(3.11)**	0.476	1.46
Di-α-methyl phenylacetic acid **(3.13)**	0.043	16

Table 3.3.2: Measurements taken for the glucose series k_d (degradation rate)

Conjugated acid to GA	$k_d (h^{-1})$	$t_{1/2}$ *(h)*
phenylacetic acid **(3.12)**	2.353 (0.628 LC-MS)	0.29 (1.5 LC-MS)
R-α-methyl phenylacetic acid **(3.11)**	0.903	0.78
S-α-methyl phenylacetic acid **(3.11)**	0.405	1.71
Di-α-methyl phenylacetic acid **(3.13)**	0.029	23.3

Table 3.3.3: Measurements taken for the GA series

Both series show the same trend; increasing α-substitution at the acyl group slows down the rate of migration (scheme 2.2.3 pg. 39). The results show that degradation and half life rates are faster for the *S*-α-methyl phenyl acetic acid **(*S*-(3.11))** and the di-α-methyl phenyl acetic acid **(3.13)** glucosides compared to the GA series. The *R*-α-methyl phenyl acetic acid **(*R*-(3.11))** and phenyl acetic acid **(3.12)** glucosides gave slower degradation and half life rates compared to the GA series. We still see the same general trend with respect to increased substitution and *(R)* and *(S)*-isomers. The phenyl acetic glucuronide derivative measurements are not certain due to solubility issues; LCMS rates have also been measured (table 3.3.3).

3.4 References

(1) Gessner, T.; Jacknowi.A; Vollmer, C. A. *Biochemical Journal* **1973**, *132*, 249-258.

(2) Yamaha, T.; Cardini, C. E. *Archives of Biochemistry and Biophysics* **1960**, *86*, 127-132.

(3) Yamaha, T.; Cardini, C. E. *Archives of Biochemistry and Biophysics* **1960**, *86*, 133-137.

(4) Sandermann, H. *Trends in Biochemical Sciences* **1992**, *17*, 82-84.

(5) Sandermann, H.; Schmitt, R.; Eckey, H.; Bauknecht, T. *Archives of Biochemistry and Biophysics* **1991**, *287*, 341-350.

(6) Xiang, J. N.; Jiang, L. H.; Chen, C. Y.; Fu, Z. Y.; Duan, J. F.; He, X. X.; Wang, K. M. *Journal of Carbohydrate Chemistry* **2006**, *25*, 595-614.

(7) Uhrig, R. K.; Picard, M. A.; Beyreuther, K.; Wiessler, M. *Carbohydrate Research* **2000**, *325*, 72-80.

(8) Wen, X. A.; Sun, H. B.; Liu, J.; Cheng, K. G.; Zhang, P.; Zhang, L. Y.; Hao, J.; Zhang, L. Y.; Ni, P. Z.; Zographos, S. E.; Leonidas, D. D.; Alexacou, K. M.; Gimisis, T.; Hayes, J. M.; Oikonomakos, N. G. *Journal of Medicinal Chemistry* **2008**, *51*, 3540-3554.

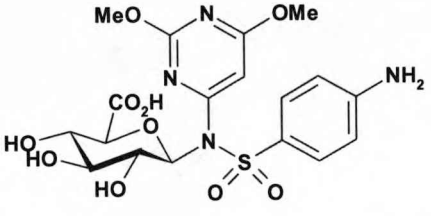
Chapter Four

N-Glucuronides and Glucosides

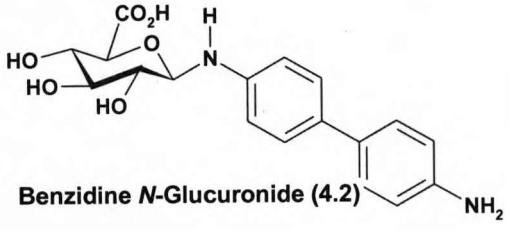
4.1 Introduction to Neutral N-Glucuronides

N-Glucuronides are formed with xenobiotics that contain amines, amides, hydroxylamines, carbamates, ureas, thioureas and sulphonamides.

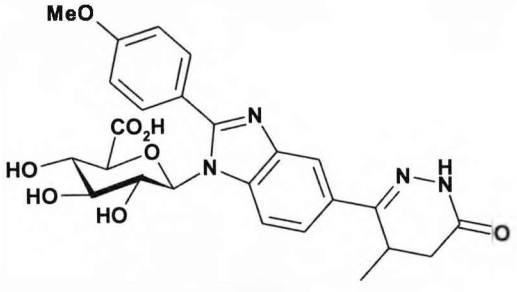
N-Glucuronidation of aryl and alkyl amines, amides, sulphonamides, and heterocyclic amines has been reported to occur *in vitro* and *in vivo* in many animal species and humans. These compounds upon glucuronidation afford a neutral *N*-glucuronide (figure 4.1.1).



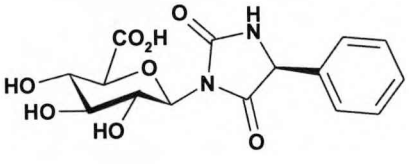
Sulphadimethoxine *N*-Glucuronide (4.1)



Benzidine *N*-Glucuronide (4.2)



Pimobendan *N*-Glucuronide (4.3)



Nirvanol *N*-Glucuronide (4.4)

Figure 4.1.1: Examples of *N*-Glucuronides, (Sulphadimethoxine[1], Benzidine[2], Pimobendan[3], Nirvanol[4], -*N*-Glucuronides)

The antiepileptic drug Retigabine[5] has two sites where *N*-Glucuronidation occurs; the primary aryl amine **(4.5)** and the secondary aryl amine **(4.6)** (figure 4.1.2). The major metabolite is that of the primary amino group; Hiller *et al.*[6] determined the levels of *N*-Glucuronide in plasma of a male volunteer and found rapid and extensive *N*-glucuronidation even 20 mins after dosing.

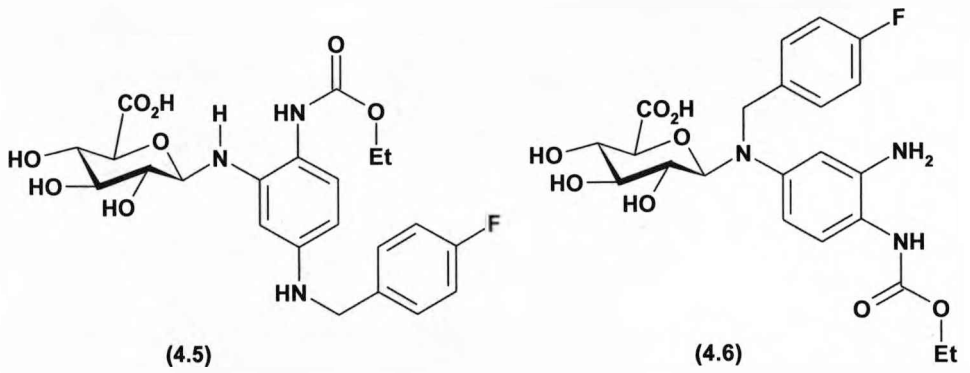


Figure 4.1.2: Two metabolites of Retigabine formed *in vivo*

4.2 Introduction to Quaternary Ammonium N^+-Glucuronides

Glucuronidation of either an aliphatic or aromatic tertiary amine group in a molecule results in a quaternary ammonium-linked glucuronide metabolite (N^+-glucuronide).[7] N^+-Glucuronidation is common for drugs such as antipsychotics (Loxapine **(4.7)**), H_1 antihistamines (Azatidine **(4.8)**), tricyclic antidepressant drugs (Amitriptyline **(4.9)**) (Figure 4.2.1), and heterocyclic containing drugs such as Midazolam **(4.10)** (scheme 4.2.2).

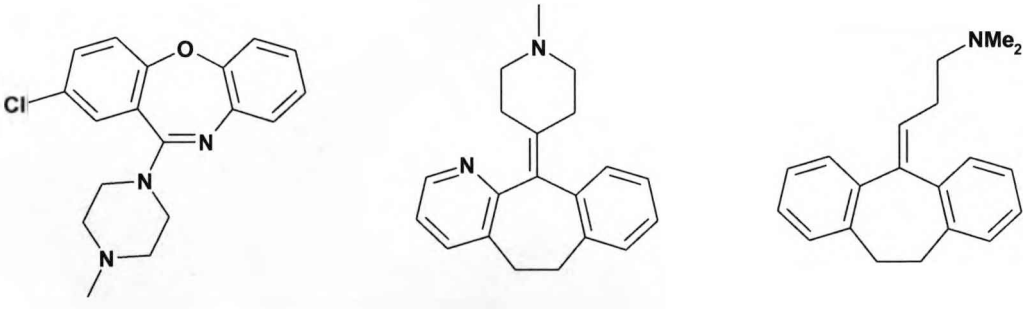
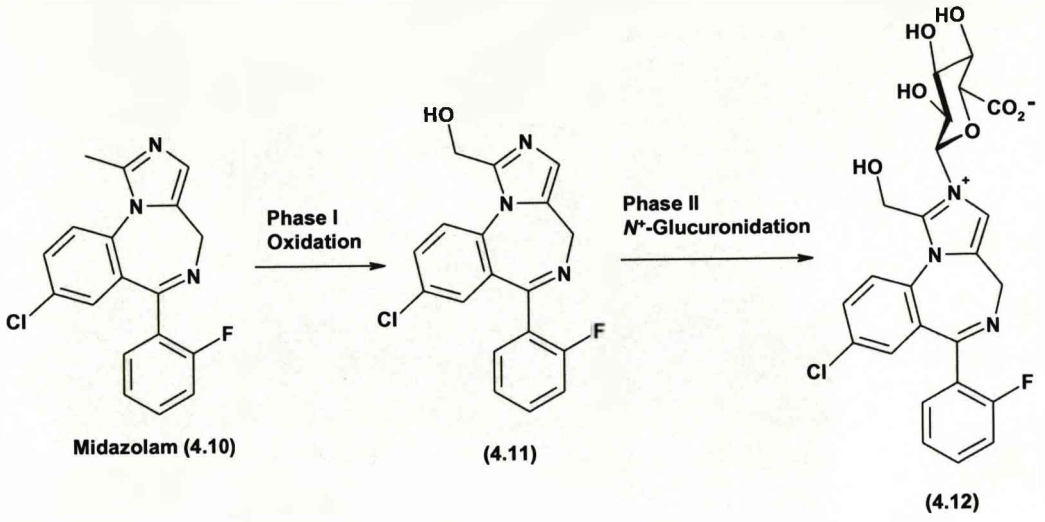


Figure 4.2.1: Loxapine **(5C)**, Azatidine **(6C)** and Amitriptyline **(7C)**

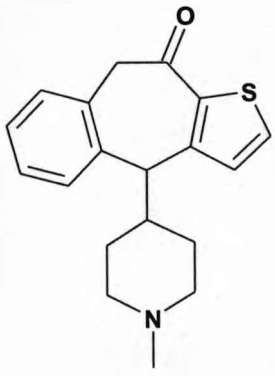
Midazolam first undergoes oxidation to 1'-hydroxymidazolam **(4.11)**, and then undergoes phase II metabolism to form the N^+-glucuronide[8] **(4.12)** (scheme 4.2.2). There has been speculation that the parent drug also undergoes direct N^+-Glucuronidation[8].



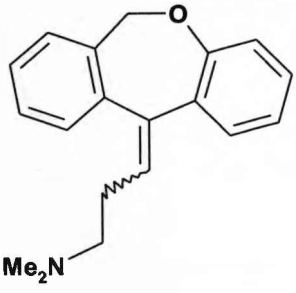
Scheme 4.2.2: Metabolism of Midazolam

There is evidence that N^+-Glucuronides are specific human metabolites[9]. It is believed that the species differences are due to the glucuronysltransferase enzymes responsible for N^+-Glucuronidation.

A further example of a quaternary N^+-Glucuronide is Ketotifen-N^+-Glucuronide[10]. Ketotifen **(4.13)** is an antiallergic drug and used for the treatment of asthma and other allergic conditions (figure 4.2.3). The seven membered ring is non-planar and so gives rise to chirality. The enantiomers differ in pharmacological potency but the drug is administered as a racemic mixture. This drug is thought to be one that gives the highest concentrations of N^+-glucuronide excreted in human urine (24%) of the initial dose. Doxepin N^+-Glucuronide (Doxepin **(4.14)**, figure 4.2.3) has also been found in high concentrations in human urine (23% of the dose).



Ketotifen (4.13)



Doxepin (4.14)

Figure 4.2.3

Species specific glucuronidation is exhibited by Ketotifen **(4.13)**[11]. In rat hepatocyte cultures, ketotifen underwent the same *N*-demethylation and *N*-oxidation reactions as those observed *in vivo*. In human hepatocytes the major metabolites were those from ketoreduction and N^+-glucuronidation. Rabbit hepatocytes also produced the N^+-glucuronide of the parent drug. This suggests that rabbits might share the same metabolic pathway as humans.

N^+-glucuronides may not always be detected in the excretory media (urine, faeces, bile) as they can be hydrolysed back to the parent drug during excretion.

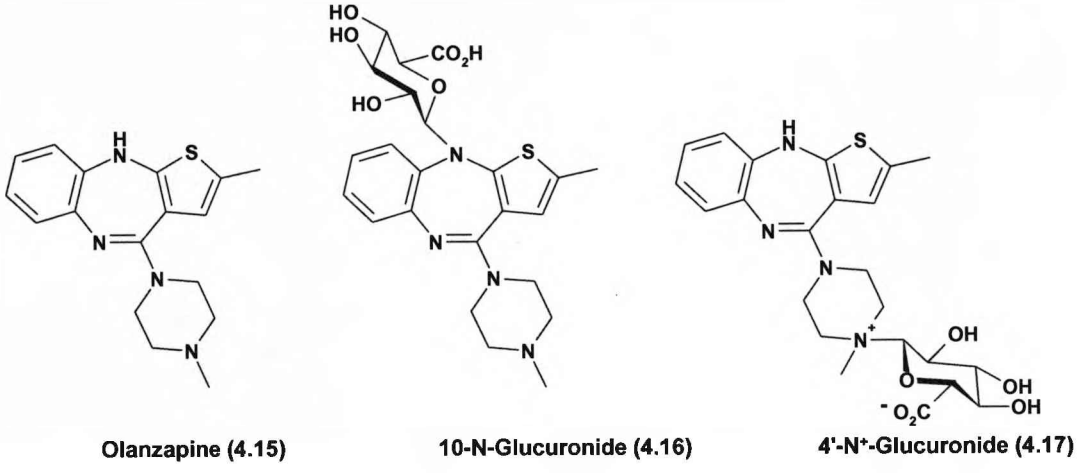
'I am of the opinion that the formation of *N*-Glucuronides is much more widespread than we have hitherto realised. In fact, there are probably several types of *N*-Glucuronides formed *in vivo* which are quite unstable.....I agree it is quite possible that the *N*-Glucuronides of drugs are formed on an appreciable scale but are not readily detectable because of their instability'

Professor R. T. Williams[12]

Although *N*-Glucuronidation is generally thought to be a detoxification process, there is evidence that quaternary ammonium compounds are associated with the release of histamine[7]. Breyer-Pfaff *et al.*[13] found that dosing of Amitriptyline-N^+-

Glucuronide **(1.11)** to a human volunteer resulted in flushing and tachycardia, with the study being terminated due to these toxic side effects.

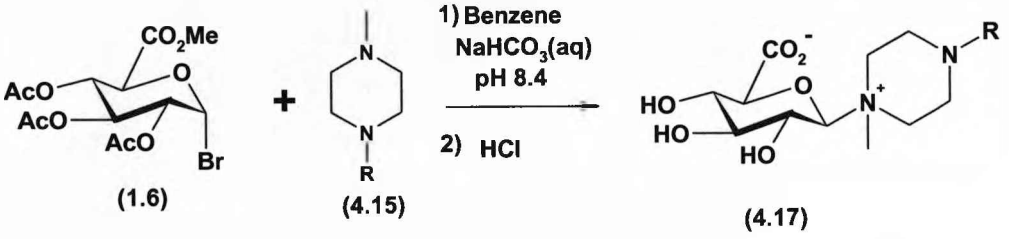
It was found by Calligaro *et al.*[14] that Olanzapine **(4.15)**, which is an antipsychotic agent, forms both Phase I and Phase II metabolites. The Phase II metabolites are conjugates with glucuronic acid. There are two sites for *N*-Glucuronidation, one being the nitrogen of the secondary amine **(4.16)** and the second through the tertiary amine of the piperazine ring **(4.17)** (Figure 4.2.4)



Olanzapine (4.15) **10-N-Glucuronide (4.16)** **4'-N⁺-Glucuronide (4.17)**

Figure 4.2.4: Olanzapine and the two *N*-Glucuronides that have been isolated from humans

Synthesis of Olanzapine N^+-Glucuronide **(4.17)** was carried out by Calligaro *et al.*[14] When they followed the procedure of Luo *et al.*[15], reacting the bromo-sugar **(1.6)** with the parent drug in a two phase system they were able to form the desired product (scheme 4.2.5) but in low yield, with a purity of 60 %. After hydrolysis of the protecting groups, they were able to isolate the product **(4.17)** but were unable to separate the impurities.



Scheme 4.2.5: Reaction conditions used by Calligaro *et al.* in the synthesis of Olanzapine N^+-Glucuronide **(4.17)**

There have been many N^+-Glucuronide metabolites isolated from nicotine, one of which is (*S*)-(-)-cotinine-N^+-Glucuronide **(4.19)** (figure 4.2.6) which forms the glycosidic bond via the pyridine ring nitrogen. Upadhyaya *et al.*[16] found that excreted nicotine-N^+-glucuronide **(4.20)** and *(S)*-(-)-cotinine-N^+-glucuronide **(4.19)** account for 3-5 % and 12-17 % respectively, of the initial dose of nicotine. Work by Caldwell *et al.*[17] confirmed that this was the major unidentified Phase II metabolite by synthesising (*S*)-(-)-cotinine N^+-glucuronide **(4.19)**.

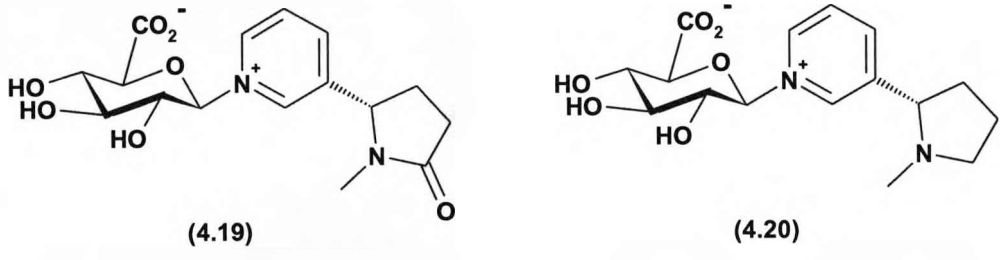
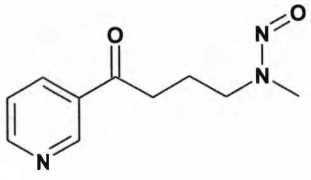
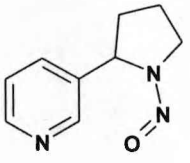


Figure 4.2.6: (*S*)-(-)-cotinine-N^+-Glucuronide **(4.19)** and nicotine-N^+-glucuronide **(4.20)**

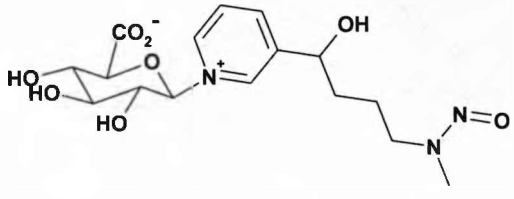
Nitrosamines, derived from nicotine, are known to be carcinogens found in tobacco products. Two that are believed to play a significant role in cancer induction in humans are 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK **(4.21)**) and *N*-nitrosonornicotine (NNN **(4.22)**) (figure 4.2.7). Upadhyaya *et al.*[16] synthesised NNK-N^+-Glucuronide and NNN-N^+-Glucuronide to try to determine whether these were significant metabolites isolated from human urine of patients using tobacco products. Later work by Wiener *et al.*[18] showed by HPLC analysis that NNAL-N^+-glucuronide **(4.23)** is a significant metabolite of nicotine.



NNK (4.21)



NNN (4.22)



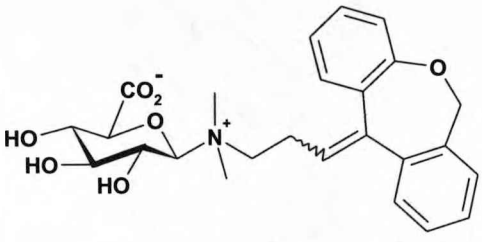
NNAL-*N*$^{+}$-Glucuronide (4.23)

Figure 4.2.7

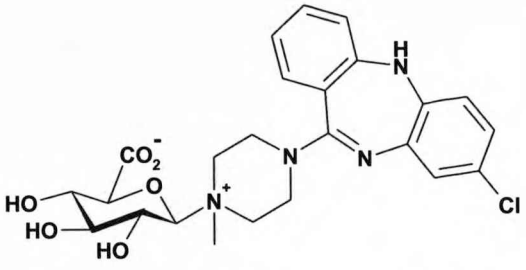
4.3 The Reactivity and Stability of N$^{(+)}$-Glucuronides and N$^{(+)}$-Glucosides

During this section I will discuss the reactivity and stability of both *N*-glucuronides and *N*-glucosides. There are differences between the neutral species originating from primary and secondary amines, and the quaternary ammonium salts with respect to stability under acidic to basic conditions.

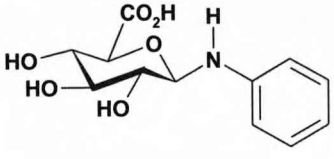
In general, the *N*-glucuronides of primary and secondary amines are unstable in acidic media. In contrast *N*$^{+}$-glucuronides are relatively stable at neutral pH and hydrolyse in basic but not acidic media.[7] This is due to there being no site for protonation on the *N*$^{+}$-Glucuronide. It was found by Hawes[7] that Doxepin *N*$^{+}$-glucuronide **(4.24)** (figure 4.3.1) was stable over the pH range of 1-10 for 3 months, whereas significant degradation occurred at pH 11 after 1 month. On the other hand Clozapine *N*$^{+}$-glucuronide **(4.25)** (figure 4.3.1), did not adhere to the general pattern of greater stability at acidic pH. Clozapine *N*$^{+}$-glucuronide **(4.25)** is fairly stable over the pH range of 4-11 however significant degradation occurred at pH 1, pH 2 and pH 3 after 3 weeks of storage, with half life times of 70, 51 and 86 days respectively.



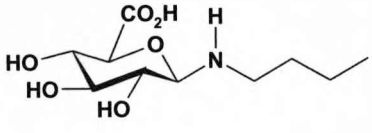
Doxepin *N*⁺-Glucuronide (4.24)



Clozapine *N*⁺-Glucuronide (4.25)



Aniline *N*-Glucuronide (4.26)

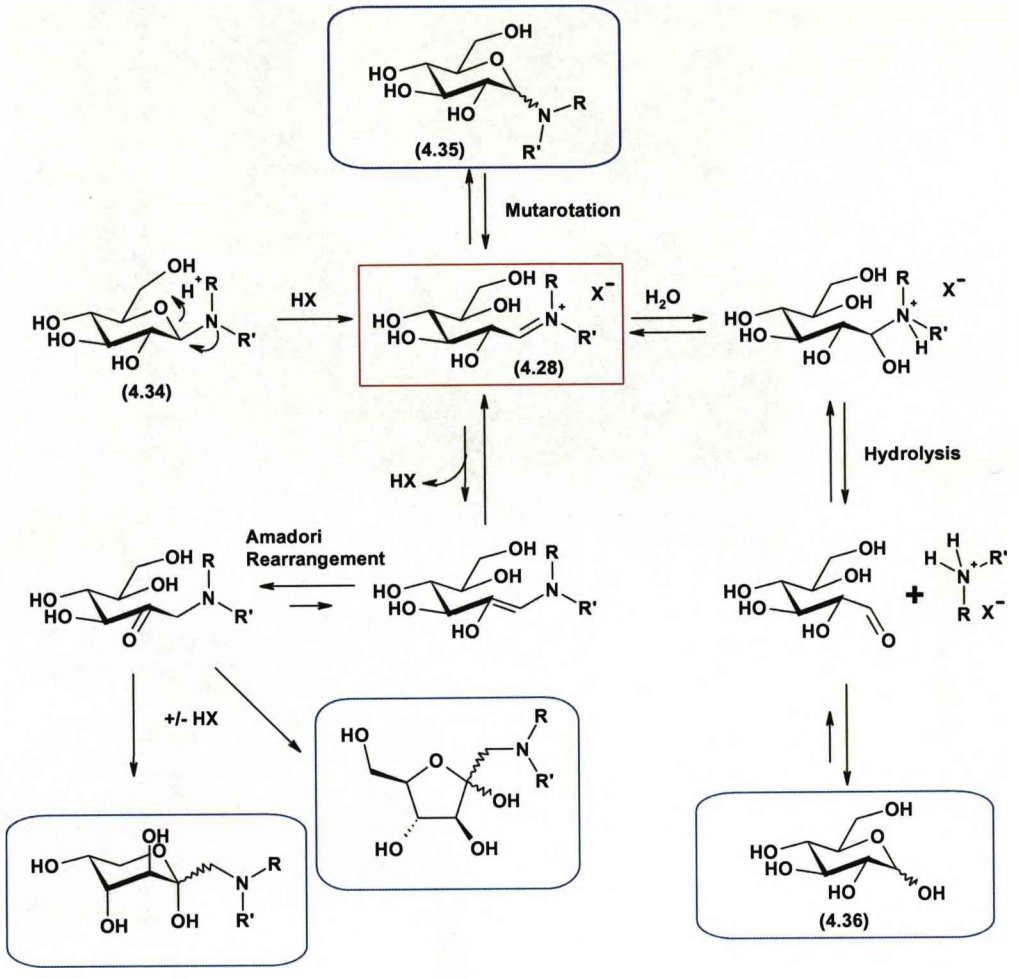


Butylamine *N*-Glucuronide (4.27)

Figure 4.3.1

Takitani *et al.*[19] synthesised various *N*-Glucuronides from D-glucuronic acid sodium salt and primary alkyl and aryl amines. They studied the dissociation of the amine and its relationship to pH and found that aniline *N*-glucuronide **(4.26)** (scheme 4.3.1) was more stable than butylamine *N*-glucuronide **(4.27)** (scheme 4.3.1) over a range of pH (4.4-7.4). This is probably due to aniline being less basic than butylamine suggesting that the dissociation goes via protonation.

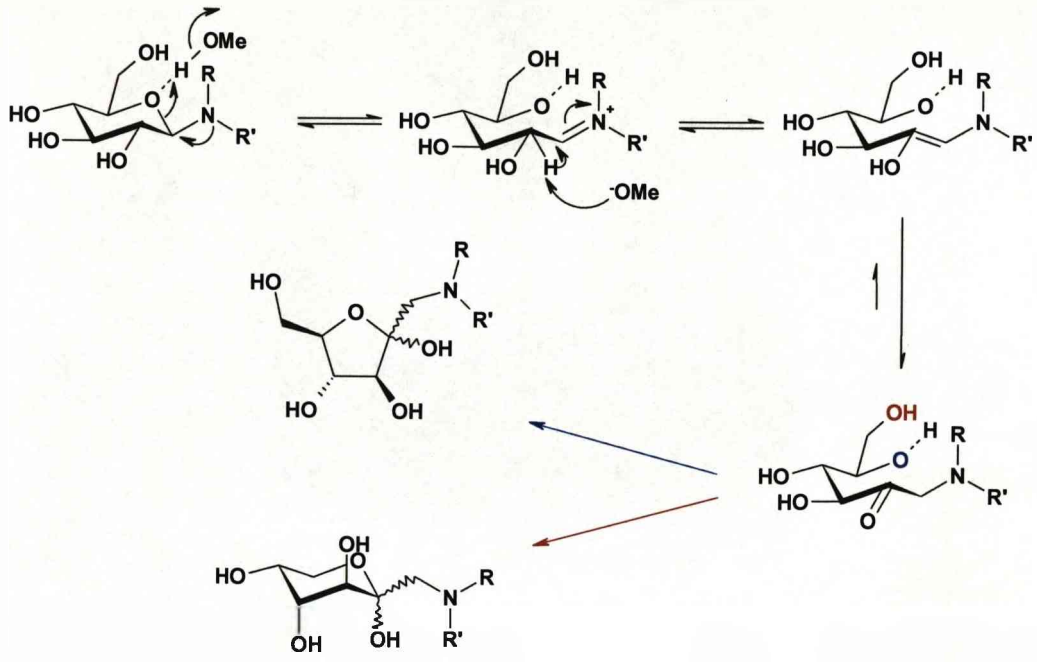
It is believed that the C-N bond of *N*-Glucuronides and *N*-Glucosides can undergo hydrolysis, mutarotation and Amadori rearrangement depending on the functional group attached to nitrogen and reaction conditions. Iminium ion **(4.28)** formation is a key step for all three processes. Once the iminium ion has formed there are several pathways that this intermediate can take (scheme 4.3.2).



Scheme 4.3.2: Mutarotation, Hydrolysis and Amadori rearrangement of *N*-Glucoside

Amadori Rearrangement

The Amadori rearrangement occurs slowly in the solid state at 25^{0}C but rapidly in hot alchoholic solution (scheme 4.3.3), showing the requirement of a mild acid catalyst. Hodge *et al.*[20] carried out some studies on the stability of *N*-glycosylarylamines and the conditions in which they undergo the Amadori re-arrangement. They found that rearrangement occurs not just with primary aryl amine *N*-glycosides but also with glycosyl derivatives of piperidine, morpholine, diethanolamine and β-phenylethylamine. The conditions under which the re-arrangement occurs is usually heating in an alcohol, but in some cases it is necessary to add ethyl malonate to promote re-arrangement[20].



Scheme 4.3.3: The Amadori rearrangement catalysed by methanol

The Amadori rearrangement is known to occur in foods[21]. The initial reaction is the condensation between a reducing sugar and either a protein or amino acid. This reaction is termed the Maillard reaction and its products are precursors responsible for aromas and flavours in many foods. Once condensation has occurred the Amadori rearrangement can take place.

Proline is one of the most important amino acids in the study of food related Maillard reactions. Its products are responsible for the aromas in roasted foods. The Amadori product, *N*-(1-Deoxy-β-D-fructopyranos-1-yl)-L-proline **(4.29)** (figure 4.3.4) has been found in many food stuffs such as liquorice root, dried apricots, and beer [21].

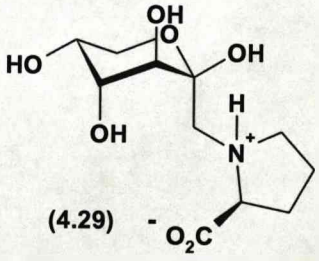
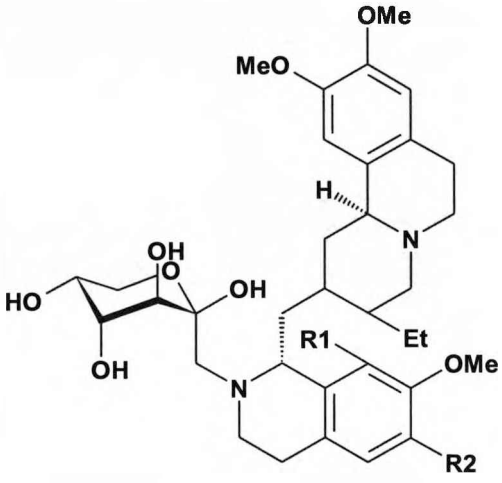


Figure 4.3.4: *N*-(1-Deoxy-β-D-fructopyranos-1-yl)-L-proline

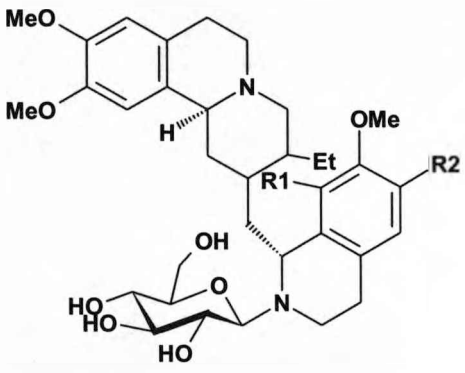
There have been Amadori rearranged type products isolated from the ipecac root[22,23]. The drug Ipecac comes from the dried roots of either *Cephaelis ipecacuanha* A. Richard or *Cephaelis acuminata* Karsten belonging to the family Rubiaceae. It has been used since the beginning of the 17th century as an emetic and expectorant, and was further used to treat dysentery in the 18th century. The Ipecac root contains many alkaloids, the main alkaloid being Emetine. Related to emetine are cephaeline and neocephaeline which were all isolated as Amadori type species, 2'-*N*-(1' '-deoxy-1' '-β-D-fructopyranosyl)emetine **(4.30)**, 2'-*N*-(1' '-deoxy-1' '-β-D-fructopyranosyl)cephaeline **(4.31)** and 2'-*N*-(1' '-deoxy-1' '-β-D-fructopyranosyl)neocephaeline **(4.32)** respectively (figure 4.3.5).



R1 = H, R2 = OMe **(4.30)**
R1 = H, R2 = OH **(4.31)**
R1 = OH, R2 = H **(4.32)**

Figure 4.3.5: Amadori products isolated from the Ipecac root

The isolation of the alkaloids from the Ipecac root involves extraction with hot methanol. Potentially Methanol can catalyse the Amadori rearrangement as already mentioned. It is therefore possible that the actual active compounds in the Ipecac root are not the rearranged compounds but compounds such as **(4.33)** (figure 4.3.6). There has been no literature evidence to suggest this.



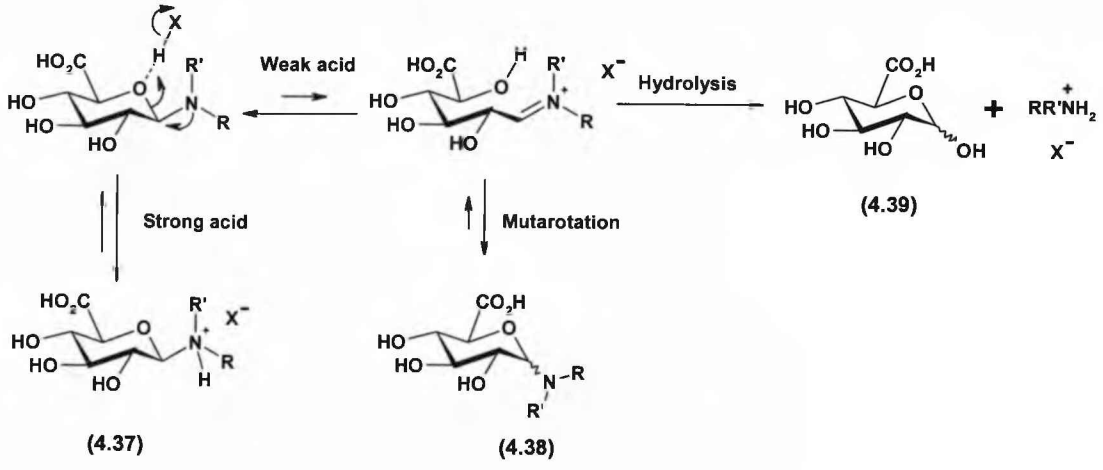
(4.33)

Figure 4.3.6: Potential products in the Ipecac route before methanol extraction

Mutarotation and Hydrolysis

Mutarotation and hydrolysis are common pathways for glucuronosylamines and glycosylamines;[24] this is dependent on the amine and the pH of the solution. There is an equilibrium between the closed sugar **(4.34)** and the iminium ion **(4.28)** where the nitrogen lone pair donates electron density into the electron deficient C1 (scheme 4.3.2 p. 88). The hydroxyl group that has been released can then re-attack C1 releasing the iminium ion. As this is not stereoselective and either face of the double bond can be attacked leading to mutarotation to give **(4.35)** (scheme 4.3.2) or **(4.38)** (scheme 4.3.7) (glucose and GA respectively). The second possibility is that an external nucleophile such as water can attack C1 and subsequently on ring closure the amine is liberated to give the sugar **(4.36)** (scheme 4.3.2) or **(4.39)** (scheme 4.3.7), and the amine.

At low pH hydrolysis is slow due to the formation of the ammonium salt **(4.37)** hence reducing the formation of the iminium ion (scheme 4.3.7). The formation of the ammonium salt also depends on the basicity of the amine; if the amine is highly basic the ammonium salt will form readily. Simon *et al.*[25] found that β-D-glucosylpiperidine is stable in 2N HCl for 17 hours at 0°C due to the formation of the ammonium salt in preference to the formation of the iminium salt.

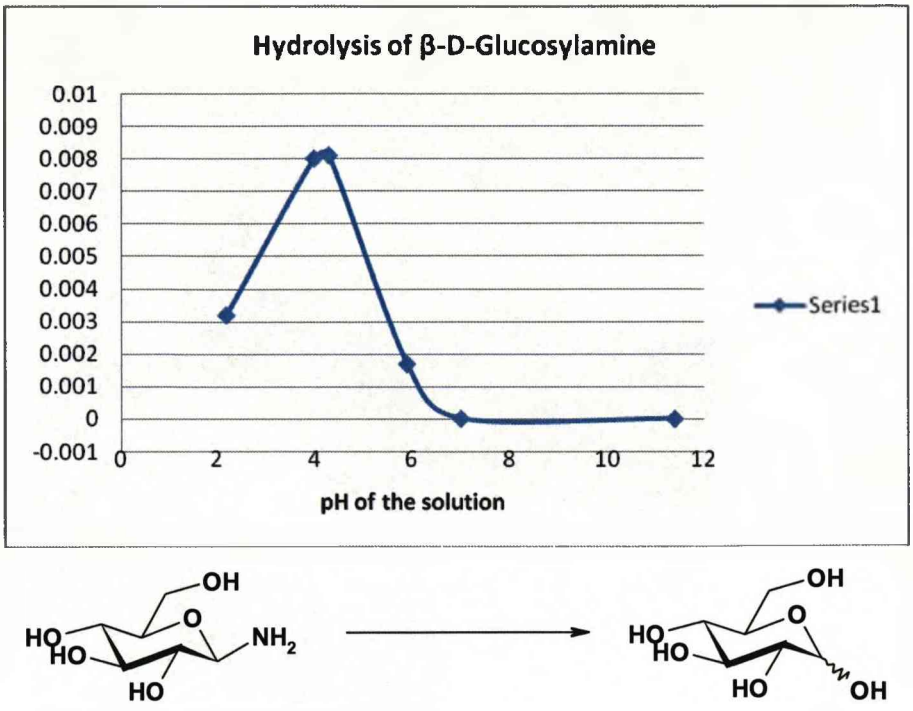


Scheme 4.3.7: The product from the glucuronysylamine is pH dependent

Isbell *et al.*[24] found that the rate of hydrolysis is very dependent on the pH of the solution. The maximum hydrolysis is obtained in weakly acidic solutions (graph 4.3.8) and this is proposed to be due to the requirement of both an acid catalyst and a hydroxyl ion. The rate constant that they found (k_{hydrol}) was calculated from the following equation:

$$k_{hydrol} = 1 / (t_2 - t_1). \log ((r_{t1}-r_\infty)/(r_{t2}-r_\infty))$$

Where t_2 and t_1 are times when a steady state has been obtained for the modifications of the amine in solution, r_{t1} and r_{t2} are the optical rotations observed at the respective times, and r_∞ is the optical rotation of the solution once hydrolysis is complete.

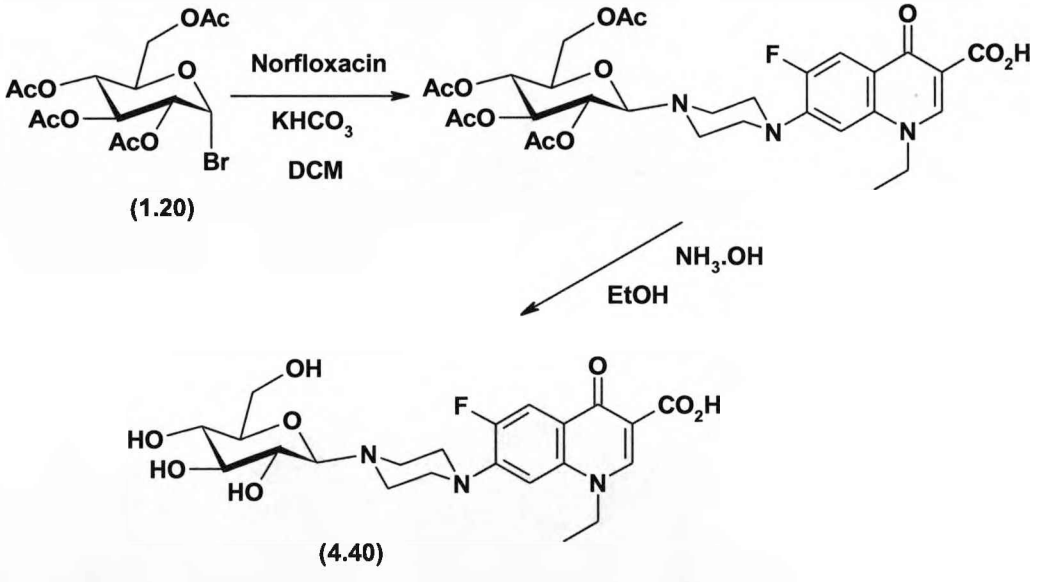


Graph 4.3.8: Values taken from Isbell *et al.*[24] k_{hydrol} vs pH of the solution which shows a maximum for hydrolysis.

Aniline derived *N*-glucuronides are known to be more stable in general than alkyl *N*-glucuronides.[26] As previously mentioned Baker[27] suggests that if the proton on the nitrogen of tetra-*O*-(acetyl)glucopyranoside aniline is replaced with a methyl group, mutarotation is not induced even in the presence of concentrated hydrochloric acid; over time hydrolysis of the amine occurs. Baker states that this outcome suggests it must be the electrostatic interaction between the proton of the acid and the ring oxygen that induces mutarotation. This induction catalyses proton transfer of *N*-glycosides that are derived from primary amines. Baker says that if the amine in the anomeric position is strongly basic, the proton from the acid co-ordinates to the nitrogen forming a salt, but this hydrolyses readily in a solution of alcohol. This suggests the ammonium salt formed from protonation is being displaced by the nucleophilic alcohol. Pigman[28] in his review agrees that glycosylamines are unstable in weakly acidic solutions (pH 4-5), but are only slowly hydrolysed by stronger acids.

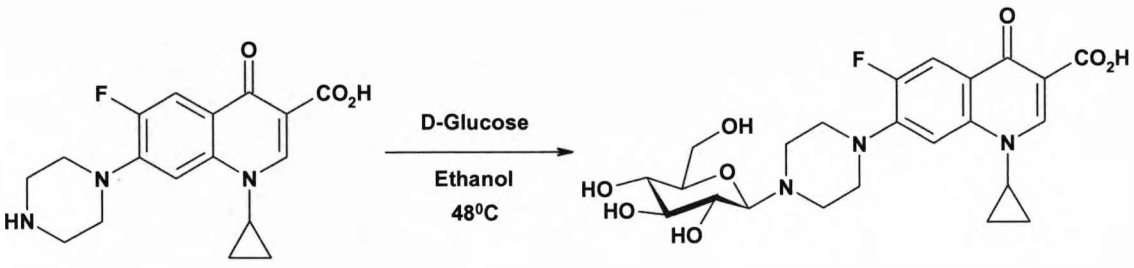
4.4 Previous Synthesis of Neutral N-Glucosides and N-Glucuronides

Secondary amine glucosides have been synthesised previously. When Zsoldos-Mady *et al.*[29] synthesised Norfloxacin-*N*-glycoside **(4.40)**, they were hoping to increase the solubility of the parent drug by addition of the sugar moiety to the antibacterial agent. They used **(1.20)** (1.1eq) and added more sugar portion wise (3 x 0.1eq) over 4 days (scheme 4.4.1). They carried out this reaction under basic conditions by adding solid potassium hydrogen carbonate. They do not state why they used basic conditions but this is probably to ensure removal of hydrobromic acid generated *in situ* that could form the hydrobromide of norfloxacin and render it unreactive, or potentially catalyse hydrolysis of the desired product.



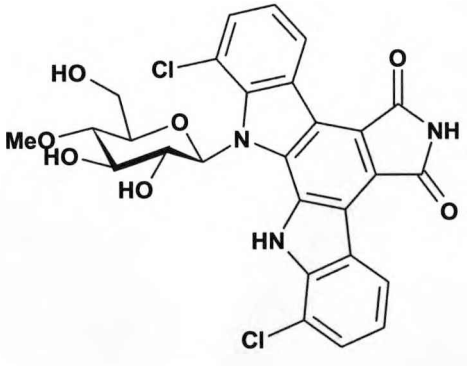
Scheme 4.4.1: Conditions for the synthesis of Norfloxacin-*N*-Glycoside

Some Ciprofloxacin derivatives have been synthesised previously by Jung *et al.*[30] (scheme 4.4.2). They took the ciprofloxacin derivative in ethanol and heated it with glucose under reflux for 48hrs, and were able to filter the product from the reaction with some methanol washes.

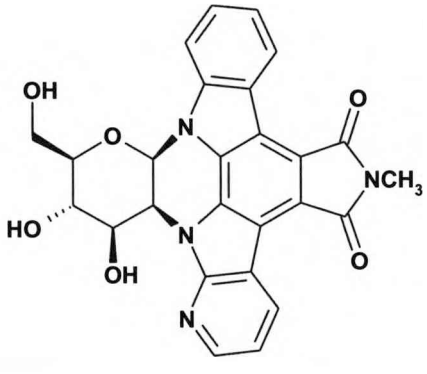


Scheme 4.4.2: Reaction of Ciprofloxacin derivative with glucose carried out by Jung *et al.*[30]

Rebeccamycin **(4.41)** is an antitumour antibiotic that possesses an indolocarbazole framework onto which is attached via a β-*N*-glycosidic bond, a 4-*O*-methoxyglucose (figure 4.4.3). Rebeccamycin's activity as an antitumour agent is due to its ability to inhibit topoisomerase I by forming a ternary DNA topoisomerase I-drug complex[31]. Messaoudi *et al.*[32] synthesised a 7-aza staurosporine analogue **(4.42)** (figure 4.4.3) which is related to Rebeccamycin and has exhibited high selectivity towards tumour cell lines tested.



Rebeccamycin (4.41)

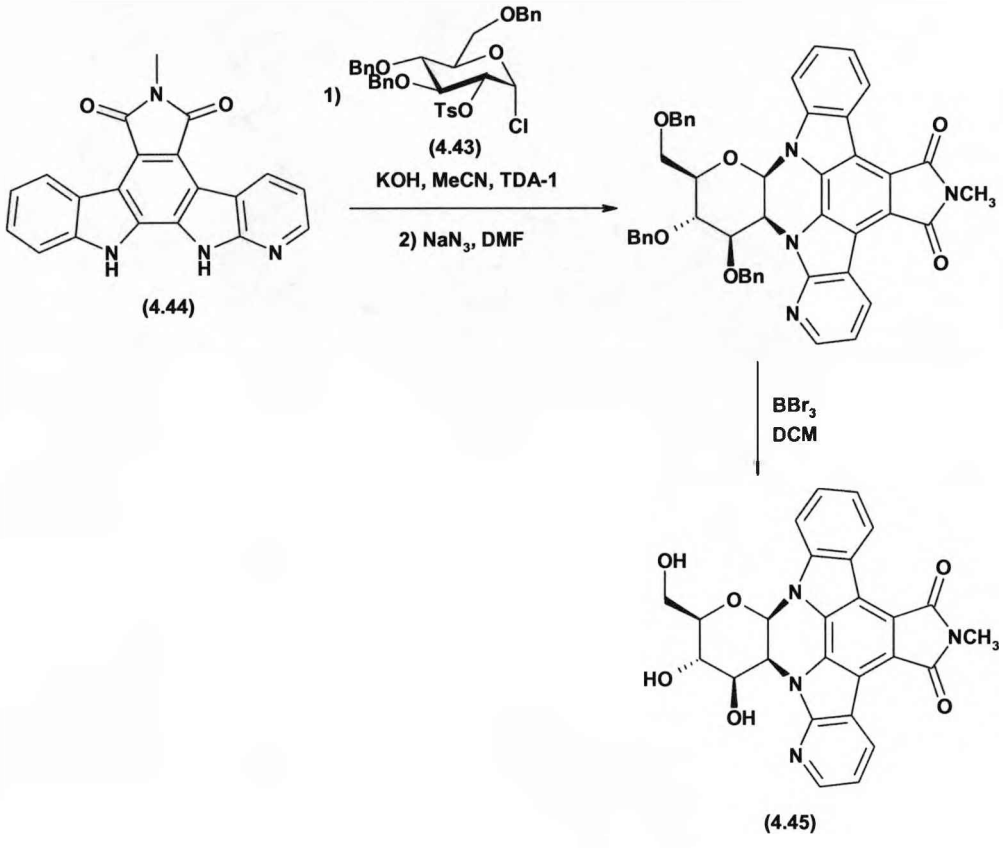


7-aza staurosporine (4.42)

Figure 4.4.3

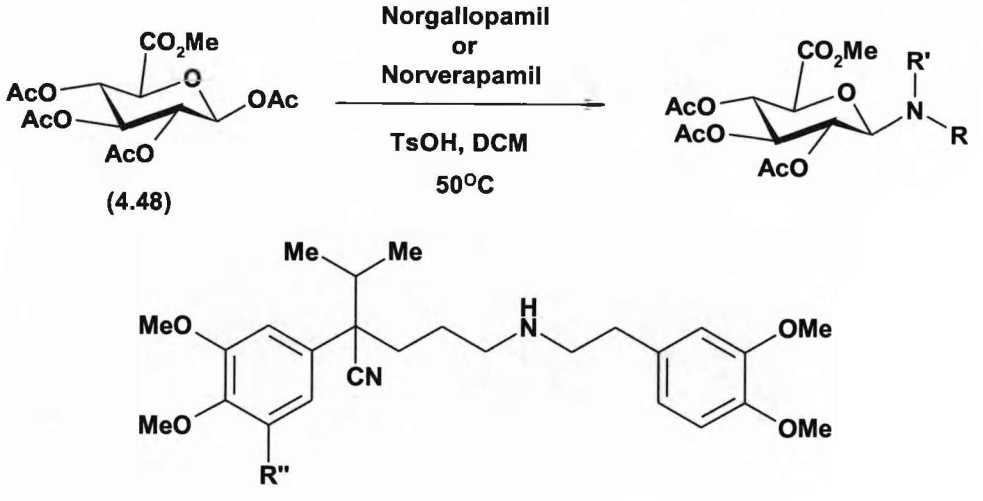
Synthesis of 7-aza staurosporine was achieved by coupling **(4.43)** with the aglycone **(4.44)** in the presence of KOH and the phase transfer catalyst tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1) (scheme 4.4.4). The next step was reaction with NaN_3 in DMF which leads to the bridged compound. The final compound

(4.45) was then isolated in the final step on subsequent deprotection of the benzyl ethers (scheme 4.4.4).



Scheme 4.4.4: Reaction conditions to synthesis a 7-aza staurosporine analogue

Gallopamil and Verapamil are two related drugs used to treat coronary heart disease. Several metabolites are formed, amongst these are the demethylated compounds Norgallopamil **(4.46)** and Norverapamil **(4.47)**, which have both been found to be excreted as their respective *N*-glucuronides. Mutlib *et al.*[33] dosed rats with Gallopamil and Verapamil and isolated the *N*-glucuronides of Norgallopamil and Norverapamil. They compared these *in vivo* samples to synthesised samples of the two *N*-glucuronides. The chemical samples were synthesised from the acid catalysed reaction of the secondary amine **(4.46** or **4.47)** and the anomeric ester **(4.48)** (scheme 4.4.5). The compounds were isolated in 15-20 % yield.



Scheme 4.4.5

4.5 Previous Synthesis of Quaternary Ammonium N^+-Glucuronides and N^+-Glucosides

Attempts to make N^+-glucuronides in the past have resulted in low yields and difficulties in isolation. Luo *et al.*[15] claimed to have synthesised a variety of aliphatic tertiary amine-containing N^+-glucuronides in a bi-phasic system (figure 4.5.1). Luo *et al.* concluded that once the N^+-glucuronide was formed it inhibited further production of the desired product when the reaction was carried out in an organic solvent such as DCM. They then designed a two phase system of water and benzene utilising $NaHCO_3$ as a phase transfer catalyst. Acetobromo-α-D-glucuronic acid methyl ester **(1.6)** is the glycosyl donor, which along with the tertiary amine is present in the benzene phase. They then proposed that once the ammonium-linked glucuronide metabolite is formed that this will pass into the aqueous phase and be deprotected to an extent by $NaHCO_3$.

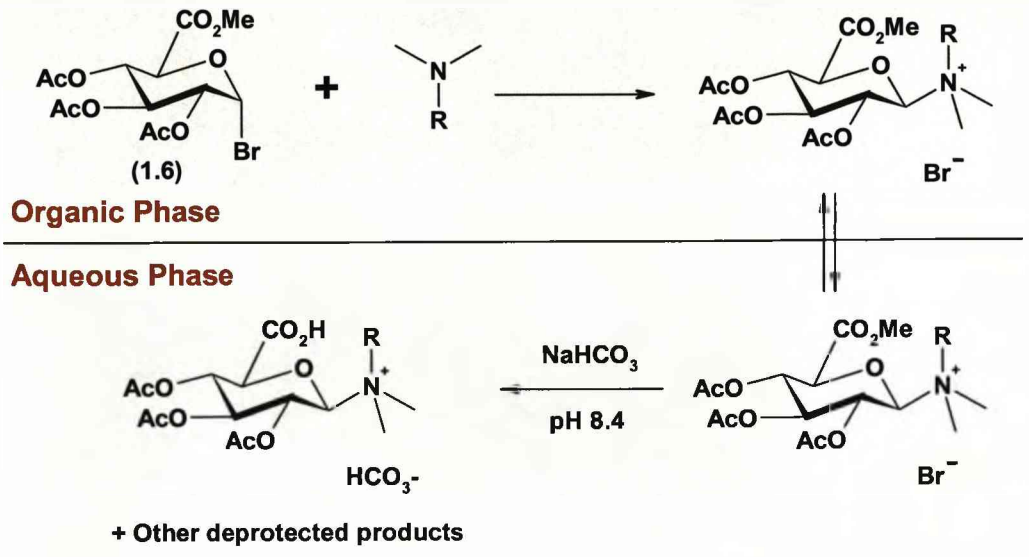
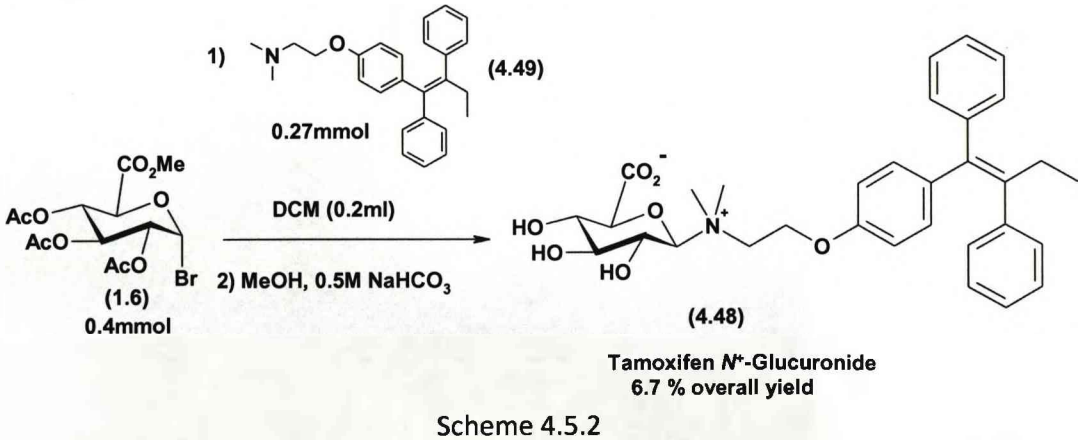


Figure 4.5.1: Bi-phase system used by Luo *et al.*

Tamoxifen N^+-Glucuronide **(4.48)** was synthesised in a direct substitution reaction between **(1.6)** and tamoxifen **(4.49)**. The intermediate was then deprotected immediately with sodium hydroxide. The overall yield for this reaction was 6.7 % (scheme 4.5.2).



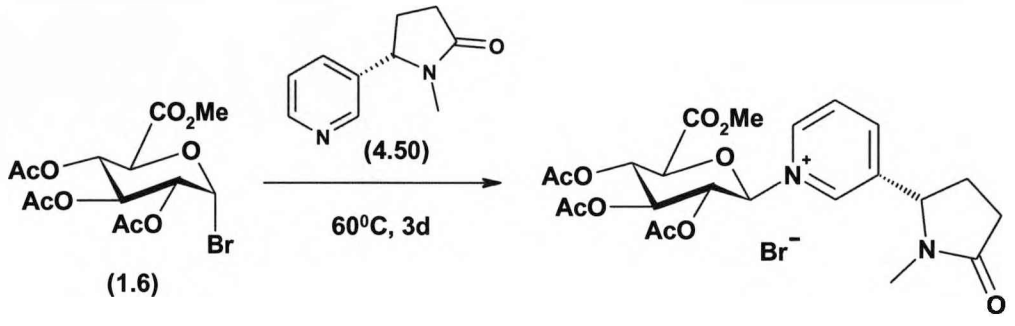
Scheme 4.5.2

Kaku *et al.*[34] used human liver microsomes fortified with UDP-glucuronic acid and TAM and were able to identify TAM N^+-glucuronide **(4.48)** by comparing it to the synthetic sample by high-performance liquid chromatography-electrospray ionization-mass spectrometry.

Tamoxifen **(4.49)** is a nonsterodial antiestrogen and is widely used for chemotherapy treatment of hormone dependent breast cancer in women. It is believed that TAM N^+-Glucuronide **(4.48)** (scheme 4.5.2) is also an active species itself and does not merely act as a detoxification agent[34].

It should be noted that others have tried the conditions of Luo *et al.* with very limited success. As already discussed, Calligaro *et al.*[14] tried the direct quaternisation of Olanzapine with **(1.6)** and found the reaction to be very low yielding, giving only 1-2 % product with a purity of 60 % (scheme 4.2.5).

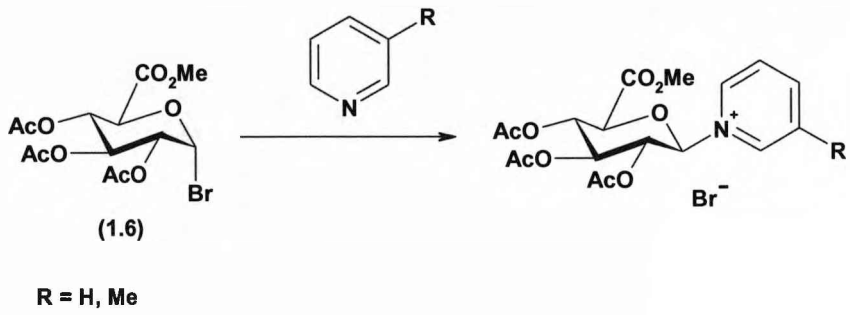
(S)-(-)-Cotinine N^+-glucuronide was syntheised by Caldwell *et al.*[17] from **(1.6)** and *(S)*-(-)-cotinine **(4.50)** by heating them together at 60^0C for 3days (scheme 4.5.3), the yield after purification was 75 %.



Scheme 4.5.3: Nucleophilic substitution of *(S)*-(-)-cotinine with bromo-sugar

Pyridinium N^+-Glucuronides appear to form more readily chemically than alkylamines during an S_N2 reaction with bromine. This is due to pyridine being less basic compared to aliphatic amines therefore inhibiting the E2 mechanism from taking place.

Dalgaard *et al.*[35] isolated substituted and unsubstituted Pyridinium N^+-Glucuronides (figure 4.5.4) with yields of 58-73 %. They reacted pyridine derivatives and **(1.6)** in the absence of solvent and isolated the product by re-crystallisation from diethyl ether.

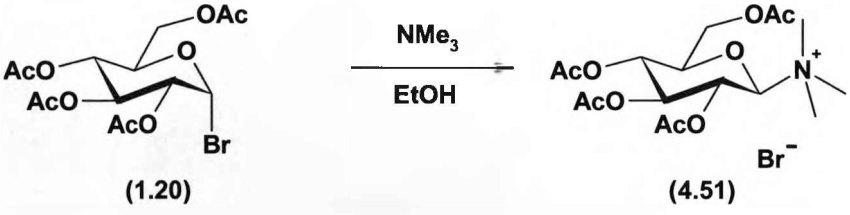


R = H, Me

Scheme 4.5.4: Pyridinium N^+-Glucuronide isolated by Dalgaard *et al.*

They found that the pyridinium compounds were stable in the solid state and in solutions below pH 10; in strongly basic solutions hydrolysis slowly occurred. They found that a 0.1M solution of pyridinium N^+-glucuronide is cleaved at a rate of 0.15mM/min at 38^0C at pH 7.

Skorupwa *et al.*[36] carried out a reaction between 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **(1.20)** and a 30 % ethanolic solution of trimethylamine (scheme 4.5.5) following the same procedure as Karrer and Smirnoff[37]. They were able to isolate the compound **(4.51)** in 30 % yield after re-crystallisation from ethanol.

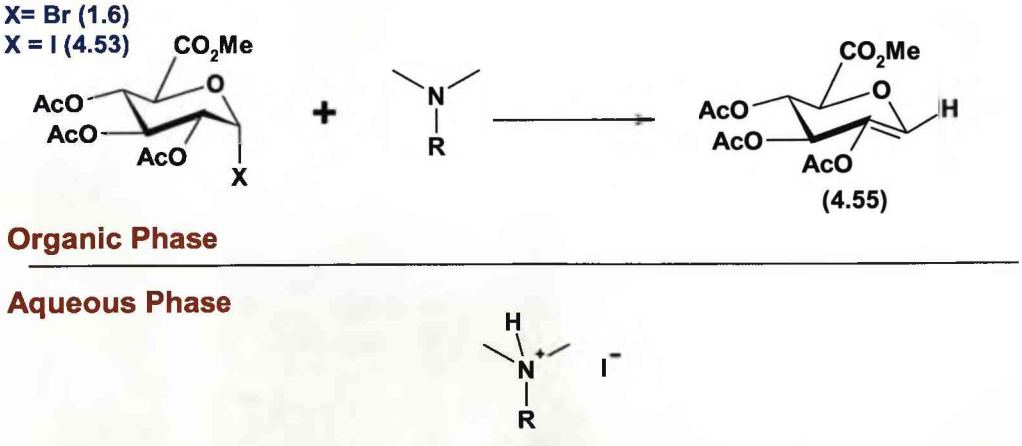


Scheme 4.5.5: Synthesis of the quaternary ammonium salt **(4.51)**

4.6 Results and Discussion part I: The Synthesis of N⁺-Glucuronides and Glucosides

-Reaction of Anomeric halides in the GA series with tertiary amines.

Our initial approach to synthesise N^+-Glucuronides involved using halide sugars, namely bromo-sugar **(1.6)** and iodo-sugar **(4.53)**. Reactions were carried out under similar conditions as Luo *et al.*[15] replacing benzene with toluene. A solution of toluene, **(1.6)** or **(4.53)** and Amitriptyline **(4.54)** was added to the aqueous sodium bicarbonate (pH 8.4). Analysis of this reaction by NMR showed that the product present in the toluene phase was actually the glucal **(4.55)**, (anomeric proton, singlet @ ~6.8ppm) and the aqueous phase contained the protonated amitriptyline (scheme 4.7.1). This suggested that **(1.6)** had undergone an elimination reaction in preference to the desired substitution reaction, showing that Amitriptyline **(4.54)** was basic enough to deprotonate the 2-H in an E2 reaction. We found the iodo-sugar **(4.53)** eliminated more readily than the bromo-sugar **(1.6)** during the reaction. There is precedence for this type of elimination reaction to occur with amines; the more basic the amine the more likely the elimination.



Scheme 4.7.1: Bi-phase reaction and the products formed

The evidence suggests that under basic conditions **(1.6)** does undergo elimination. A reaction carried out by Kaji *et al.*[38] to give the eliminated product **(4.55)** used diethylamine and tetrabutylammonium bromide (1.5:1.0 eq) in DMF. Lergenmuller

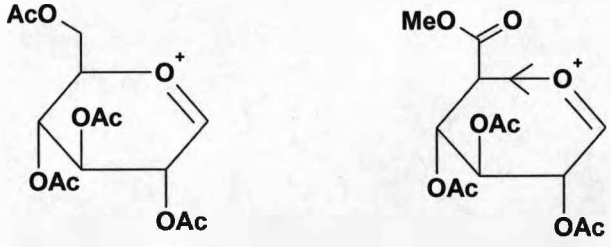
and Lichtenthaler[39] formed the glucal **(4.55)**, from **(1.6)** using dimethylamine in the presence of tetrabutylammonium bromide in DMF.

Our next approach was to carry out the direct nucleophilic substitution reaction using solvents of varying polarity as it is known that increasing the polarity of the reaction solvent can favour S_N2 over E2[40]. The solvents that we tried were, ethanol, dimethylformamide, acetone, isopropanol, tetrahydrofuran and dioxane, but changing the solvent had no effect, and we still saw no evidence by NMR of any S_N2 reaction.

We also looked at reactions with no solvent. We heated the bromosugar **(1.6)** and Amitriptyline **(4.54)** to 60°C to form a 'melt', but we only saw degradation from this reaction.

-Reaction of anomeric bromine in the glucose series with tertiary amines

The reaction was then carried out on the corresponding glucose derivatives to see if the electronics of the ring system would influence the nucleophilic substitution. If the reaction went via an S_N1 mechanism this would mean that the oxonium ion intermediate **(4.56)** would be more stable in the glucose series (scheme 4.7.2). This is due to increased electron withdrawing ability of the methyl ester in **(4.57)** which de-stabilises the oxonium ion.

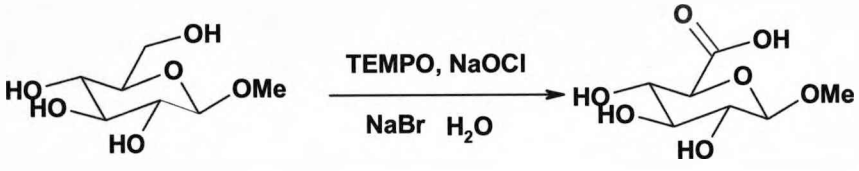


(4.56) **(4.57)**

Figure 4.7.2: Oxonium ion intermediate of both the glucose series **(4.56)** and glucuronic acid series **(4.57)**

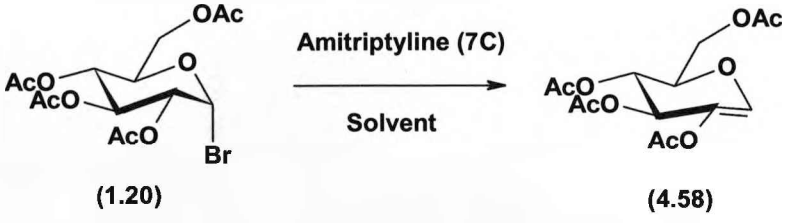
If the reaction was successful in the glucose series, the glucose derivative could then be oxidised in the final step via a TEMPO oxidation reaction to give the corresponding glucuronic acid product.

Baisch *et al.*[41] carried out this reaction on α and β-glucosides. They used TEMPO to catalyse the oxidation of the CH_2OH group in water containing the oxidant sodium hypochlorite (scheme 4.7.3).



Scheme 4.7.3: Reaction conditions to oxidise C^6

A solution of **(1.20)** in acetone was used in the nucleophilic substitution reaction with amitriptyline **(4.54)**; again elimination was preferred over substitution (scheme 4.7.4). Changing the solvent to EtOH, as Skorupowa *et al.*[36] (scheme 4.5.5) do in their reaction (pg 94) with trimethylamine was also tried, but this also gave elimination over substitution.



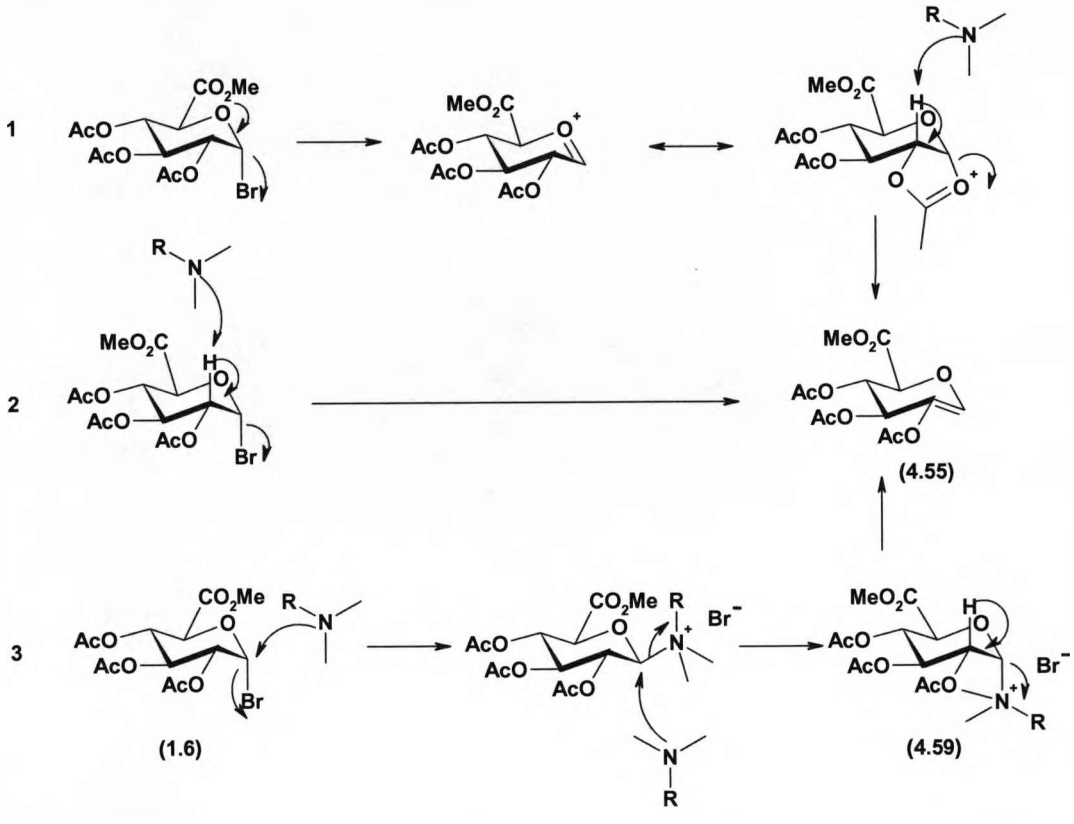
(1.20) **(4.58)**

Solvents used: Acetone, DMF, THF, and EtOH

Scheme 4.7.2: Elimination reaction

Summary for the halide activated sugars and tertiary amines

There are several possible mechanisms for the generation of the eliminated products **(4.55)** (scheme 4.7.1) and **(4.58)** (scheme 4.7.2). Amitriptyline may deprotonate the 2-position of the sugar with bromine leaving at the same time (E2) (scheme 4.7.3, route 2). Alternatively the mechanism could proceed via E1, in which case the bromine would leave first, creating a carbocation; this is a secondary carbocation that can be stabilised via the oxonium ion and neighbouring group participation of the acetate protecting group (scheme 4.7.3, route 1). Generally this type of intermediate is only formed in the presence of a promoter such as Ag (I) salts. This same carbocation would also be formed if the reaction were to proceed via S_N1. There is a third possibility in which the desired product is formed via S_N2, but in the presence of excess Amitriptyline **(4.54)** it could undergo another substitution reaction to give the α-anomer **(4.59)** which can then eliminate by Hofmann degradation (scheme 4.7.3, route 3). Recent experiments have ruled out the possibility for Hofmann degradation, as we see no elimination of the β-quaternary product in the presence of the amine.



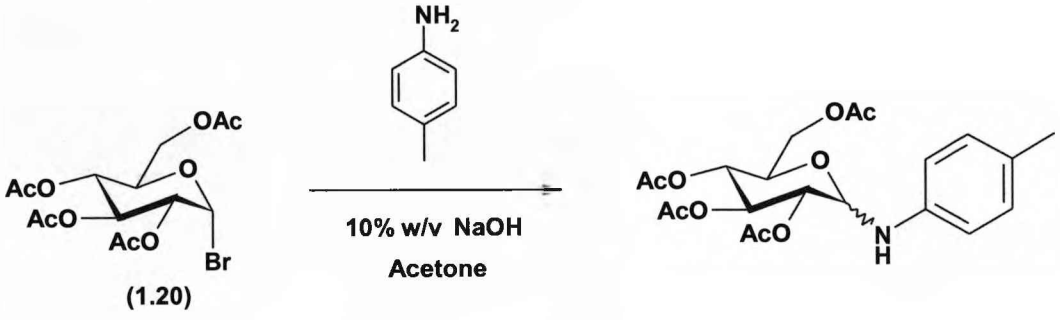
Scheme 4.7.3: Possible mechanisms to form the elimination product: **1:** E1, **2:** E2, **3:** Hofmann degradation

Following these unsatisfactory early results, we decided it was necessary to look at the reaction of primary and secondary amines with the bromosugar in both the glucose **(1.20)** and glucuronic acid series **(1.6)**. We hoped to arrive at a better understanding of the reactivity of amines with sugar electrophiles, and seek alternative ways of introducing nitrogen at the anomeric position.

Reaction of the Anomeric Bromide with Primary Amines

Bognar *et al.*[26] synthesised various aniline substituted *N*-glycosides. They carried out the reaction of *p*-toluidine with **(1.20)** under basic conditions (sodium hydroxide 10 % w/v) (scheme 4.7.4). They purified the material with several re-crystallisations which gave them an isolated yield of 10 %. They were able to separate the α and β anomers during re-crystallisation, with the β-anomer being the major component

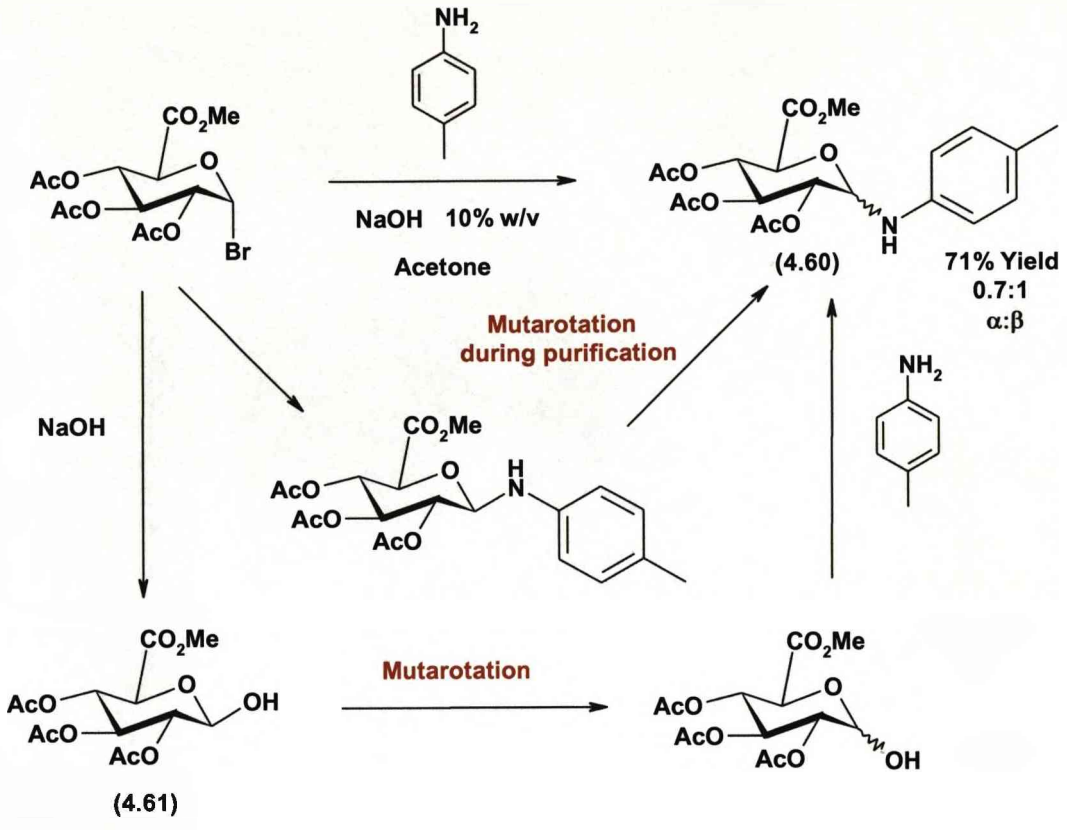
isolated (92 % β-anomer and 8 % α-anomer). Aniline derivatives have a pKa of ~5, much weaker than the bases we have looked at. Therefore it is less likely that elimination would occur using aniline derivatives.



Scheme 4.7.4: Reaction conditions to form *p*-toluidine-*N*-glucoside

We carried out the same reaction in the glucuronic acid series and we were able to isolate the protected *p*-toluidine-*N*-glucuronide **(4.60)** in 71 % yield as a mixture of α and β anomers (scheme 4.7.5). We found that the material contained 0.7:1 α/β-anomers; unlike Bognar *et al.*[26] we used silica gel column chromatography to purify the material. We would expect the initial reaction between the α-bromo-sugar **(1.6)** and toluidine to be S_N2 and therefore give inversion at the anomeric centre. It is plausible that the acidic nature of the silica during purification was able to induce mutarotation. As discussed in the introduction, Baker *et al.*[27] found that glycosylamines derived from primary amines undergo mutarotation in weakly acidic environments, catalysed by the protonation of the pyranose oxygen.

Recently during our investigations of N^+-Glucuronide synthesis we have found amines can undergo a condensation reaction with the hemiacetal form of the sugar. This suggests that a possible role of the NaOH in this reaction is actually to form the hemiacetal **(4.61)**, which then undergoes a condensation reaction with the *p*-toluidine to give a mixture of anomers (scheme 4.7.5).



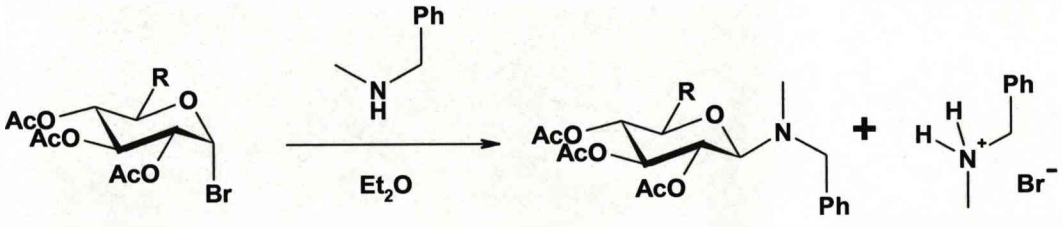
Scheme 4.7.5: Reaction of toluidine with the bromo-sugar under basic conditions

Reaction of the Anomeric Bromide with Secondary Amines

Baker[27] found that secondary amines react with **(1.20)** in an S_N2 fashion. He isolated various *p*-substituted benzylmethylamine *N*-glycosides. He used two equivalents of amine; one to undergo the substitution reaction and the second to form the HBr salt of the second equivalent of amine *in situ*.

We utilised this chemistry and carried out the same procedure with both **(1.6)** and **(1.20)** and methyl-N-benzylamine (scheme 4.7.6). The HBr salt of the amine is insoluble in diethyl ether and can be filtered off from the reaction mixture. The glucose derivative **(4.62)** was isolated by re-crystallisation in 29 % yield; the glucuronic acid derivative **(4.63)** proved more troublesome and re-crystallisation was not possible. Purification of the GA series could probably be achieved by column chromatography, with some hydrolysis. Purification was not carried out

due to stability worries at the time of carrying out these reactions. Both series gave only the β-product by NMR analysis, suggesting that the reaction proceeds via S_N2 with no mutarotation.



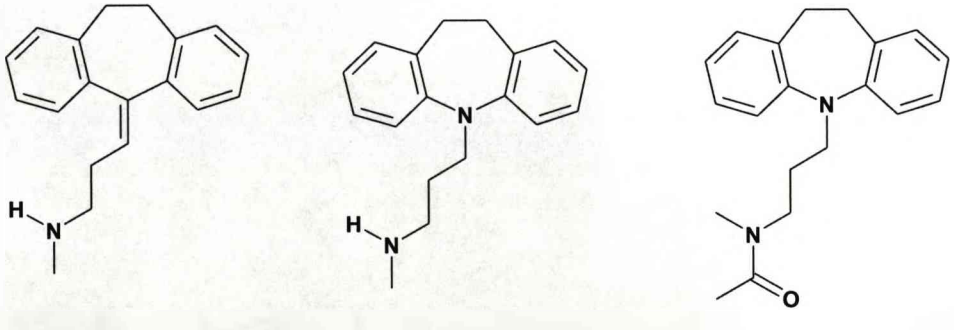
R = CO_2Me, (1.6)
R = CH2OAc, (1.20)

R = CO_2Me, (4.63)
R = CH2OAc, (4.62)

Scheme 4.7.6: Reaction conditions from Baker[27] to form tertiary anomeric amines in the glucose and glucuronic acid series

Drug Examples using Anomeric Bromide in the Glucose and Glucuronic Acid Series

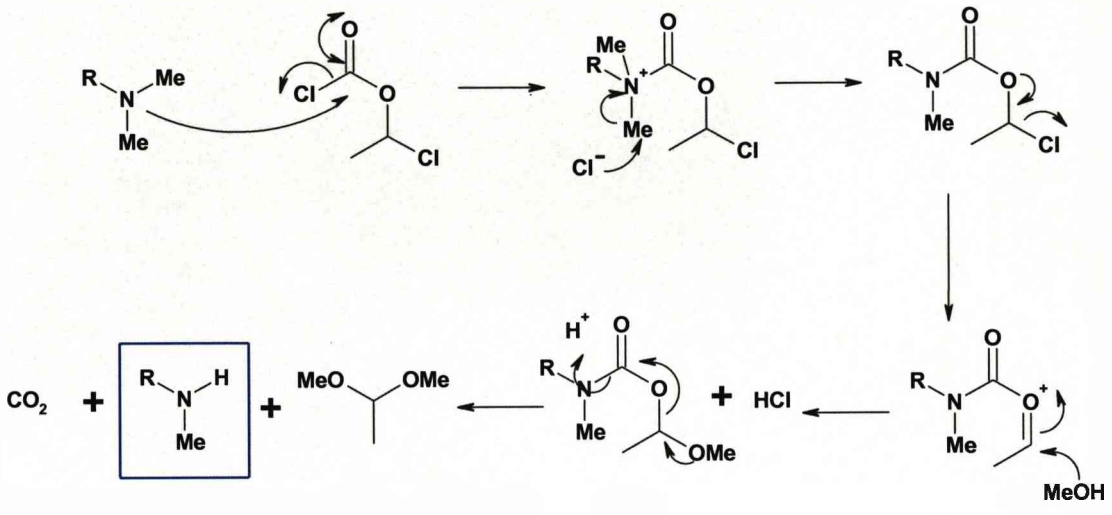
Following the promising result using N-methlbenzylamine, we moved on to look at Nortriptyline **(4.64)** and Desipramine **(4.65)** (figure 4.7.7) (the des-methyl versions of Amitriptyline and Imipramine respectively). Our ultimate aim was to synthesise examples of drug N^+-glucuronides.



(4.64) (4.65) (4.66)

Figure 4.7.7: Nortriptyline, Desipramine and acetylated desipramine

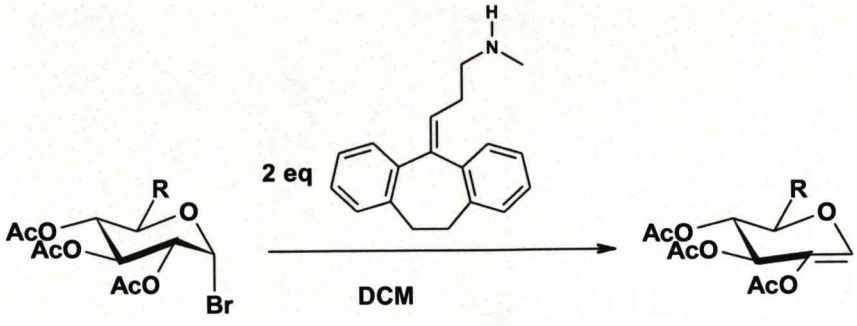
Most N-methylated tertiary amino drugs which we were interested in are commercially available as their des-methyl derivative, but demethylation can be achieved using the Olofson reaction[42] (scheme 4.7.8).



Scheme 4.7.8: The Olofson reaction to form secondary amines from tertiary amines with a simple alkyl group

Nortriptyline

The conditions used for the reaction of **(1.6)** and **(1.20)** with methyl-*N*-benzylamine (scheme 4.7.6) did not give product when using Nortriptyline **(4.64)** (scheme 4.7.9). LCMS and NMR analysis showed that elimination was taking place (scheme 4.7.9), but no substitution was observed. As Nortriptyline **(4.64)** is poorly soluble in diethylether, we were limited to using DCM as the solvent for the reaction. The reaction carried out using methyl-*N*-benzylamine is favourable due to the insolubility of the methyl-*N*-benzylamine.HBr, which drives the equilibrium of the reaction to the desired product.



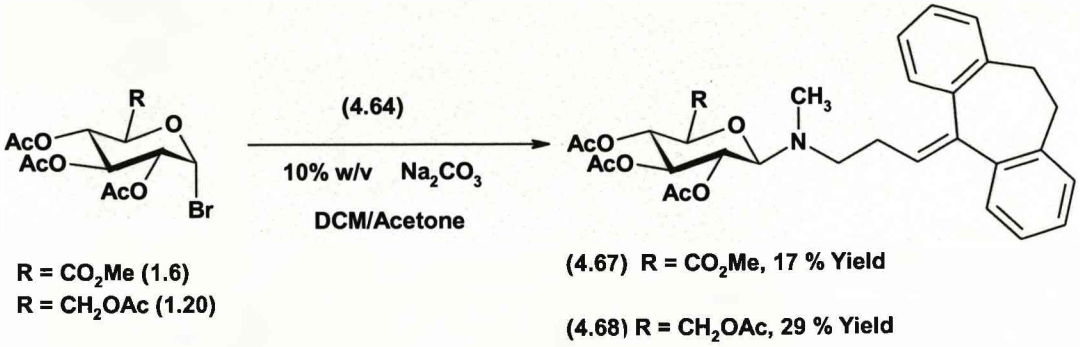
R = CO_2Me (1.6) or CH_2OAc (1.20)

Scheme 4.7.9

Again, we had problems with the E2 side reaction due to the basic nature of nortriptyline.

We then tried similar conditions to those developed by Bognar *et al.*[26] who added 10 % w/v sodium hydroxide to the reaction mixture (scheme 4.7.4, pg 102). We used a mixture of acetone and DCM as solvent for solubility reasons. This improved the reaction and we were able to isolate the desired products **(4.67)** and **(4.68)** in 15 % and 22 % yield (scheme 4.7.10). We looked at other bases such as 10 % w/v sodium bicarbonate and 10 % w/v sodium carbonate; the latter gave slightly improved yields in both the glucose and GA series. Column chromatography was used to purify the compounds, and we isolated products **(4.67)** and **(4.68)** in low yield (17 % for the GA series and 29 % for the glucose series).

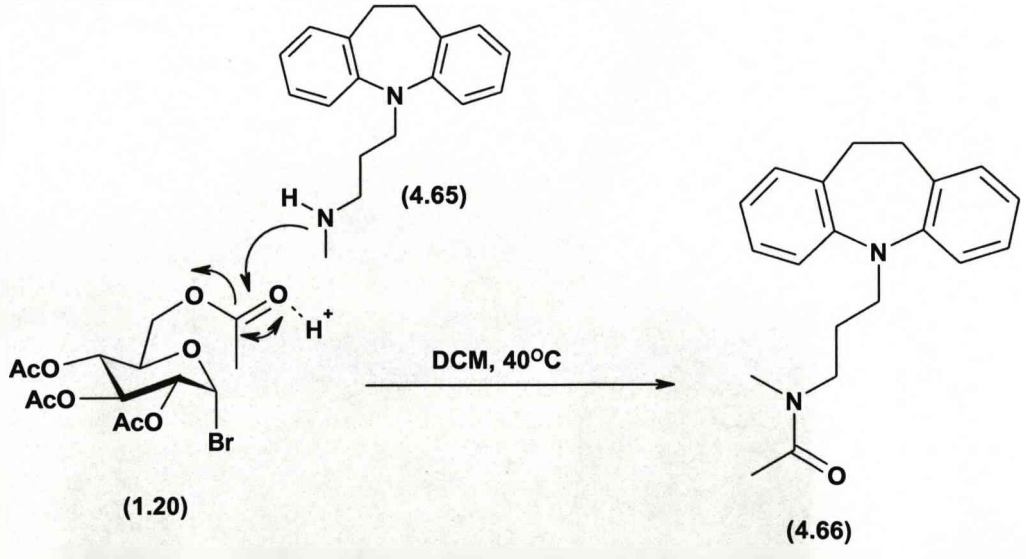
NMR analysis of the crude reaction mixture suggested elimination of the bromine was occurring, as well as hydrolysis in both series. We also saw significant amounts of starting materials remaining in both reactions. We suspect that hydrolysis on the silica during column chromatography reduced our yields further.



Scheme 4.7.10

Desipramine

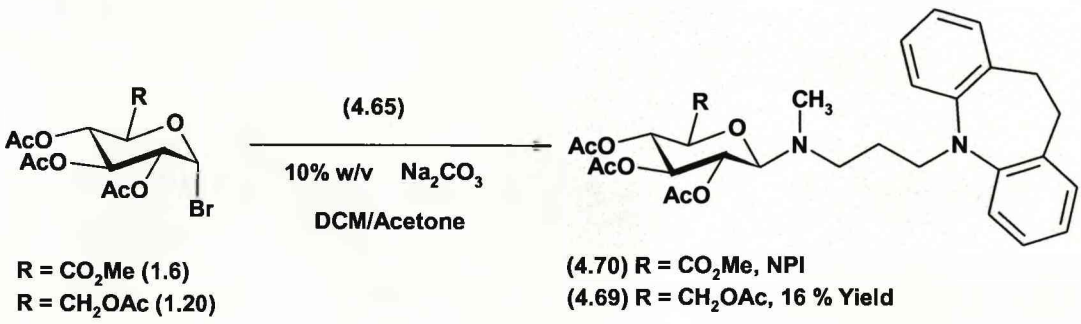
The reaction of desipramine **(4.65)** under neutral conditions with the bromo-sugar in the glucose series **(1.20)** gave some product but in low yields as shown by NMR of the reaction mixture. The major component of this reaction was the acetylated desipramine **(4.66)** (figure 4.7.7). The mechanism by which acetylation occurs is thought to be acid catalysed (scheme 4.7.11).



Scheme 4.7.11: Possible mechanism for the amide formation

The reaction was then carried out with 10 % w/v Na_2CO_3(aq) added to the reaction; this reduced the amount of acetylated desipramine **(4.66)** generated, giving the product **(4.69)** in 16 % yield after purification (scheme 4.7.12) (preparative HPLC

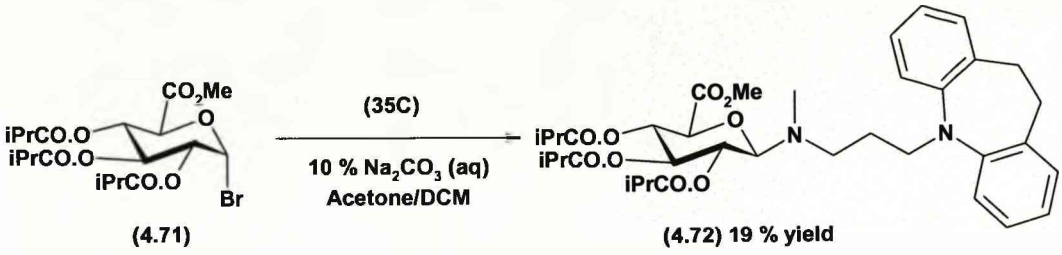
was required to remove the E2 by-product). This suggests that the hydrobromic acid from the initial substitution reaction was catalysing amide formation (scheme 4.7.11) when no base was present.



Scheme 4.7.12

The reaction of desipramine **(4.65)** and the glucuronic acid bromosugar, **(1.6)** showed little product **(4.70)** by LCMS, even when using 10 % w/v Na_2CO_3(aq) in the reaction. The main component isolated from the reaction was the acetylated desipramine **(4.66)** (figure 4.7.11). This could be due to the slower reactivity of the glucuronic acid series compared to the glucose series; the glucose series is known to be more reactive at the anomeric position[43].

To eliminate the amide formation we then looked at a more hindered protected sugar **(4.71)**[44] (scheme 4.7.13). We chose isobutyrate ester protecting groups, as we believed that deprotection could still be achieved under mild conditions. Although amide formation was not observed during our investigations, our yields (~19 %) were not improved to any extent due to the other by-products (elimination, hydrolysis). It was also difficult to fully remove the eliminated by-product from the desired product.



Scheme 4.7.13

The labile nature of the intermediate products on silica gel **(4.67)** and **(4.68)** was shown by TLC. Samples of pure isolated product **(4.67)** and **(4.68)** were loaded onto a TLC plate that was left for one hour before running. This showed the parent nortriptyline **(4.64)** and the respective 1-hydroxy sugars **(4.73)** and **(4.61)** (figure 4.7.14). We investigated the use of alumina for purification, but this proved to be unsuitable as the resolution was very poor.

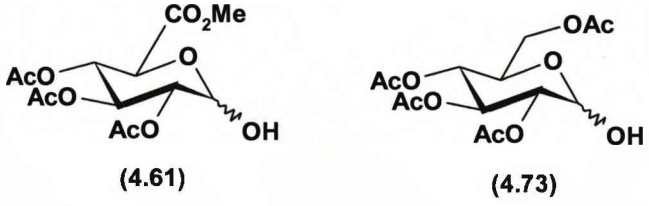


Figure 4.7.14: Product from hydrolysis during the reaction and purification

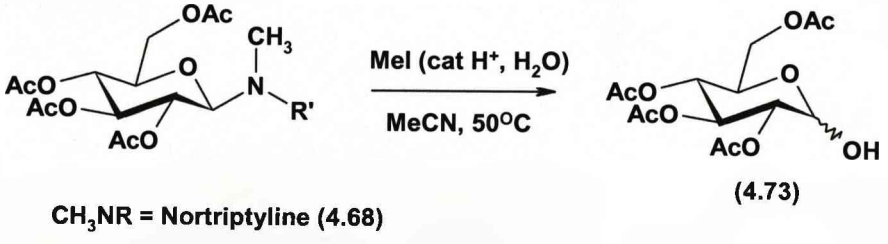
Summary

The successful reaction of *p*-toluidine with bromo-glucose **(1.20)** and glucuronic acid derivative **(1.6)**, compared to unsuccessful reactions with alkyl amines (Nortriptyline **(4.64)** and Desipramine **(4.65)**) is thought to be due to *p*-toluidine being a weaker base, therefore reducing the elimination mechanism. Stability of the product is also a factor; it is generally believed that sugar-aniline derivatives are more stable again, due to the lower basicity of the nitrogen.[45]

Quaternisation of the Nitrogen to form Quaternary Ammonium Salts

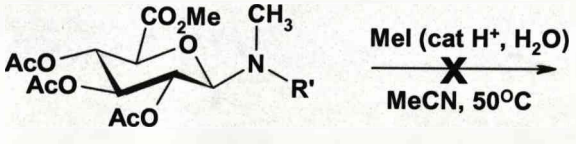
Once the Nortriptyline-*N*-Glucoside/glucuronide **(4.67, 4.68)** were isolated, reaction with a methyl electrophile was required to form the quaternary ammonium salt. Nortriptyline-*N*-glucoside **(4.68)** proved troublesome. We used methyl iodide as the electrophile and carried out the reaction in dry acetonitrile. The reaction was followed by LCMS and after 6 hours there were no signs of product, but the parent amine could be seen suggesting hydrolysis (scheme 4.7.15). It is probable that the methyl iodide used had catalytic traces of H^+ and H_2O that could cause hydrolysis. We then distilled methyl iodide and carried out the reaction with this neutral source but found no reaction occurred (scheme 4.7.15).

Methyliodide was also used in the attempted quaternisation of Nortriptyline-*N*-glucuronide **(4.67)**. There was no reaction and no hydrolysis seen, showing how much more robust to mild acidic conditions the glucuronide series is compared to the glucose series (scheme 4.7.15).



CH_3NR = Nortriptyline (4.68)

(4.73)

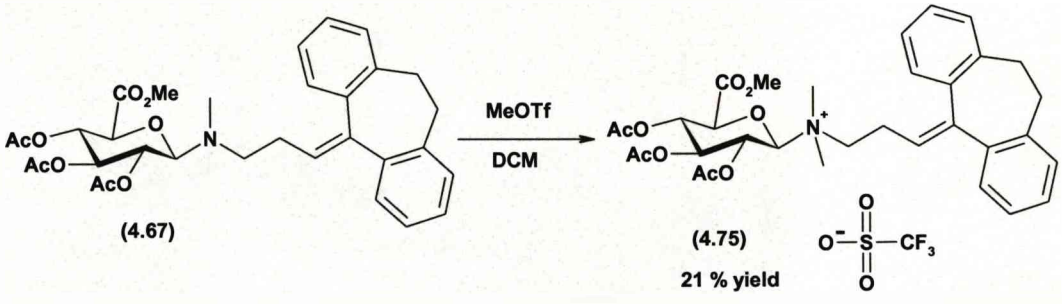


CH_3NR = Nortriptyline (4.67)

Scheme 4.7.15: Reactions of **(4.68)** and **(4.67)** with mildly acidic methyl iodide

We then looked at the reaction of methyl triflate with the GA series intermediate **(4.67)**. The initial experiment was carried out at 40^0C but the reaction proceeds just as efficiently at room temperature (scheme 4.7.16). Attempted recrystallisation of

the crude solid resulted in decomposition. The product **(4.75)** was isolated by column chromatography and eluted in 10 % IPA/DCM giving the product in 21 % yield.



Scheme 4.7.16: Conditions for quaternisation of **(4.67)** with methyltriflate

We wanted to probe the reactivity of the nitrogen to see if we could use a less reactive methyl electrophile than methyl triflate. We investigated methyl tosylate and methyl nosylate (figure 4.7.17) in an attempt to quaternise **(4.67)**, but found that neither gave us the desired product.

Methyl tosylate **Methyl nosylate**

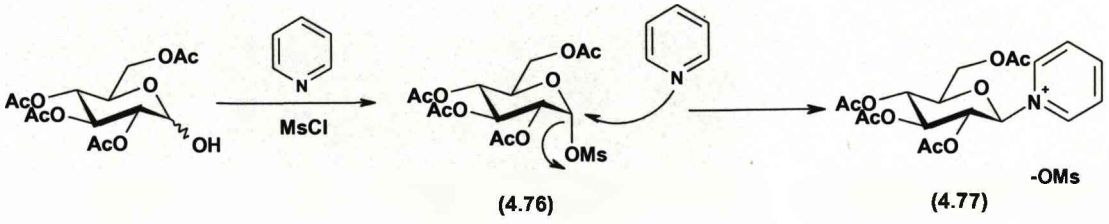
Figure 4.7.17

As our yields were unsatisfactory during the investigations of anomeric bromides we wished to investigate alternative anomeric leaving groups with the aim of reducing or eliminating the undesired E2 reaction.

Anomeric Sulphonates and their Reaction with Tertiary Amines

We decided to investigate anomeric sulphonate leaving groups in the glucose series, hoping that the more reactive glucose series would give us direct quaternisation of a tertiary amine.

Leaving groups can determine the mechanism of a reaction, and it is thought that tosylate leaving groups favour S_N2 over E2[40]. We first looked at the anomeric mesylate **(4.76)**, in the glucose series. Our first attempts to synthesise the mesylate sugar employed pyridine as base and methanesulfonyl chloride (scheme 4.7.18).

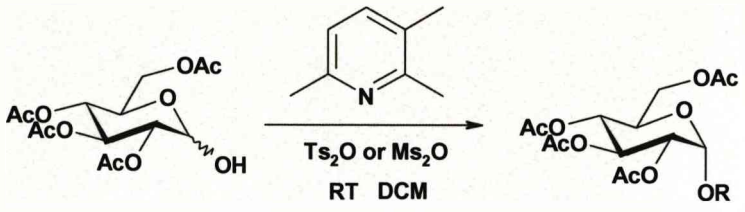


Scheme 4.7.18: Attempted synthesis of the mesylate sugar

This reaction proved unsuccessful; we reasoned that once the mesylate sugar was formed, pyridine acted as a nucleophile and generated the pyridinium-N^+-Glucuronide **(4.77)** (scheme 4.7.18). Mass spectrometry (MS) and NMR analysis suggested that the α-chlorosugar had also been isolated by column chromatography.

The synthesis of mesylate sugars has been carried out in the past by Leroux *et al.*[46] who used the mesylate sugar **(4.76)** to generate the anomeric bromo-sugar *in situ*. They used 2,3,6-collidine as base, which is non-nucleophilic, and methanesulfonic anhydride, which does not generate any nucleophilic counter ions.

We were able to isolate both the tosylate **(4.78)** and mesylate sugars **(4.76)** as the pure α-anomer using the same reaction conditions as Leroux (scheme 4.7.19).

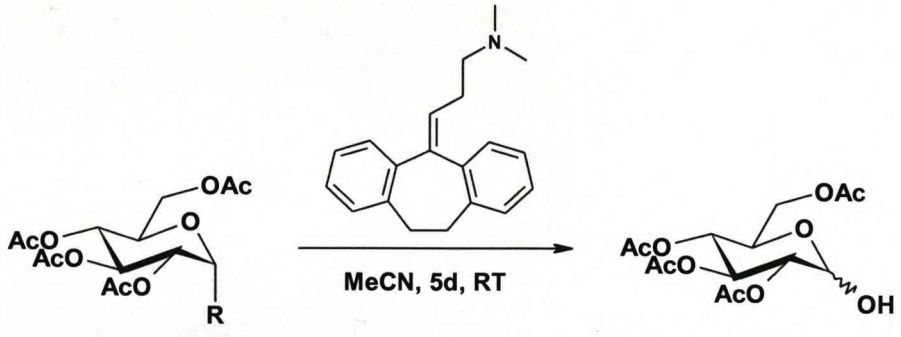


R = (4.76) Ms, 29 % Yield
(4.78) Ts, 22 % Yield

Scheme 4.7.19: Reaction conditions to synthesise the anomeric sulfonates

The mesylate sugar **(4.76)** was then reacted with Amitriptyline **(4.54)**, in acetonitrile for 5d at room temperature. Analysis of the crude reaction by NMR showed that the mesylate sugar had hydrolysed to the hemiacetal **(4.73)** (figure 4.7.20).

The tosylate sugar **(4.78)** also hydrolysed and gave no desired product (scheme 4.7.20). We saw no elimination of the sulphonate sugars as we had seen with the halide sugars which seemed promising.

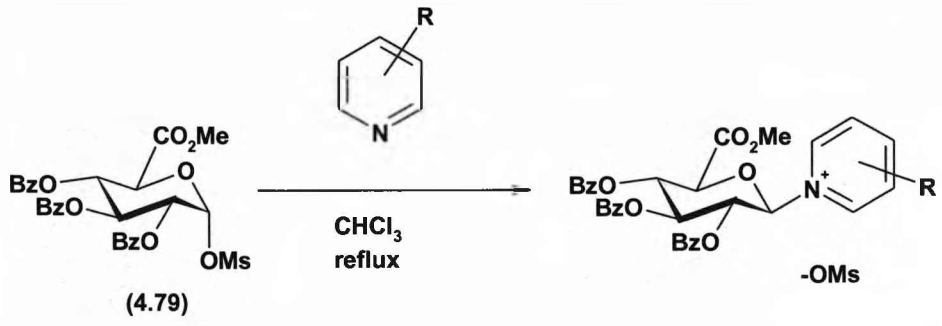


R = OMs (4.76) or OTs (4.78)

(4.73)

Scheme 4.7.20

Since we looked at using anomeric sulphonates, Araya *et al.*[47] published their work on the synthesis of N^+-Glucuronides using 2,3,4-tri-*O*-benzoyl-1-methanesulfonyl-α-D-glucopyranuronate **(4.79)** (scheme 4.7.21).



Scheme 4.7.21

Although they were successful in their synthesis when using pyridine derivatives, imidazole derivatives, isoquinoline and 1-methylbenzimidazole, they were unsuccessful when using primary, secondary and tertiary amines (figure 4.7.22). They state that the reaction gave complex mixtures, agreeing with our findings.

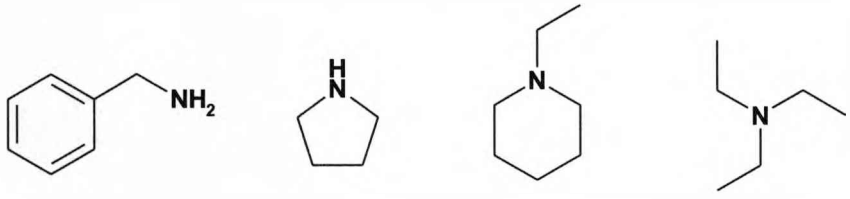
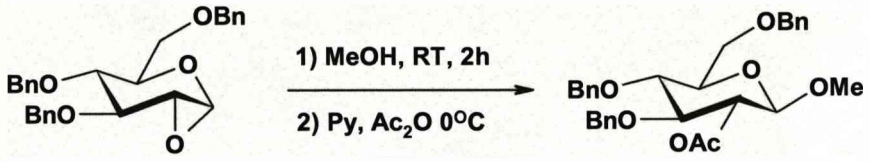


Figure 4.7.22: Amines used during the reaction with the mesylate sugar that gave complex mixtures

1,2-Anhydrosugars (epoxides) and their Reaction with Tertiary Amines.

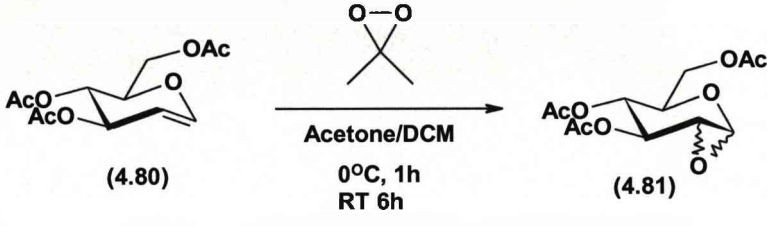
Due to the labile nature of the sulfonate sugars we decided to look at 1,2-anhydrosugars. We knew that there was no possibility for elimination using an epoxide, and hoped that the strained epoxide ring would open readily when reacting with a tertiary amine.

Epoxides are known to react well in substitution reactions with nucleophiles, and using them should eradicate the problem of elimination. Halcomb and Danishefsky[48] have used epoxides in their work predominately to make glycosides (scheme 4.7.23).



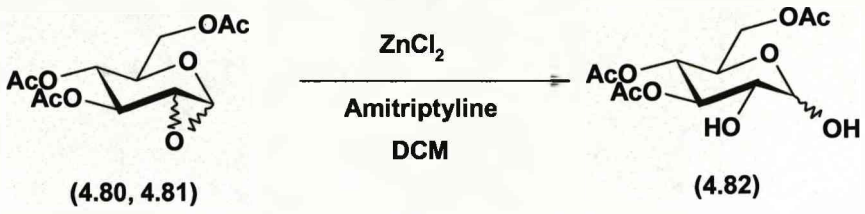
Scheme 4.7.23: Reaction carried out by Halcomb and Danishefsky[48] to synthesise glycosides from epoxide sugars

The commercially available starting material, 3,4,6-tri-*O*-acetyl-D-glucal **(4.80)** and dimethyldioxirane were used to make the 1,2-anhydro sugar following the procedure of Cheshev *et al.*[49]. Dimethydioxirane (DMDO) was generated from acetone, water, $NaHCO_3$ and Oxone. The DMDO was then distilled from the reaction flask as a solution in acetone and then added to a solution of **(4.80)** in DCM, giving quantitative formation of **(4.81)** (scheme 4.7.24). The formation of the epoxide was followed by TLC, and was used without any purification.



Scheme 4.7.24: Reaction conditions to make the 1,2-anhydro sugar

The epoxide **(4.81)** was then reacted with Amitriptyline **(4.54)** (2 eq) in the presence of $ZnCl_2$ (1M in Et_2O) (1eq). On analysis by NMR it appeared that the Amitriptyline had not reacted with the epoxide. We were only able to isolate the hydrolysed sugar **(4.82)** from the reaction (scheme 4.7.25) and we suspected the presence of polysaccharides from the initial NMR analysis.

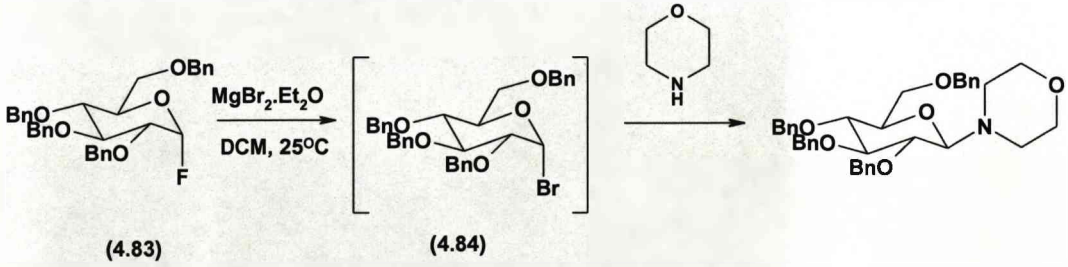


Scheme 4.7.25

Changing protecting groups

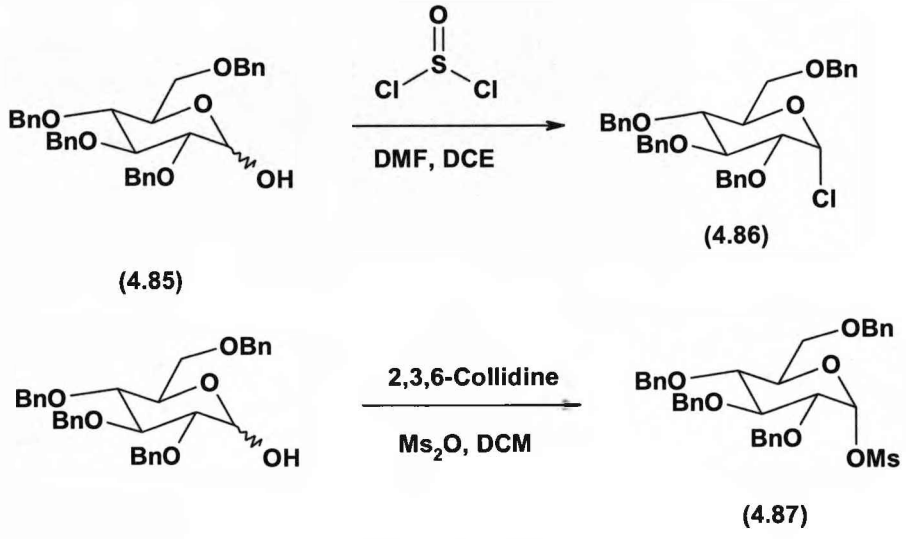
We then decided to look again at the direct quaternisation of tertiary amines with anomeric halides, but with a change of protecting group. Up to this point we had only looked at ester protecting groups that are known to be electron withdrawing. We hoped that by changing to a more electron donating group that we would render the 2-H less acidic, and we could limit the elimination reaction. The other advantage of using more donating protecting groups is that they enhance the reactivity of C1 in substitution reactions.

Nicolaou *et al.*[50] used the benzyl protected glycosyl fluoride **(4.83)** in the S_N2 reaction with morpholine. They used $MgBr_2.Et_2O$, which they claim forms the more reactive glycosyl bromine **(4.84)** *in situ* (scheme 4.7.26). It is possible that the activated benzyl protected sugars are more susceptible to the S_N2 reaction, and as they state, the bromosugar **(4.84)**, if formed is extremely reactive in this series. Benzyl protected glucose are termed the 'armed' series, whereas acetylated sugars are termed the 'disarmed' series due to their electron donating or withdrawing effects respectively[51].



Scheme 4.7.26: Proposed intermediate from the reaction carried out by Nicolaou *et al.*

From the commercially available 2,3,4,6-tetra-*O*-benzyl glucopyranoside **(4.85)** we synthesised the cholorosugar **(4.86)** and the mesylate sugar **(4.87)**, (scheme 4.7.27) although the mesylate sugar seemed quite unstable. The bromosugar **(4.84)** in this series is so unstable that it cannot be isolated, but only reacted immediately.

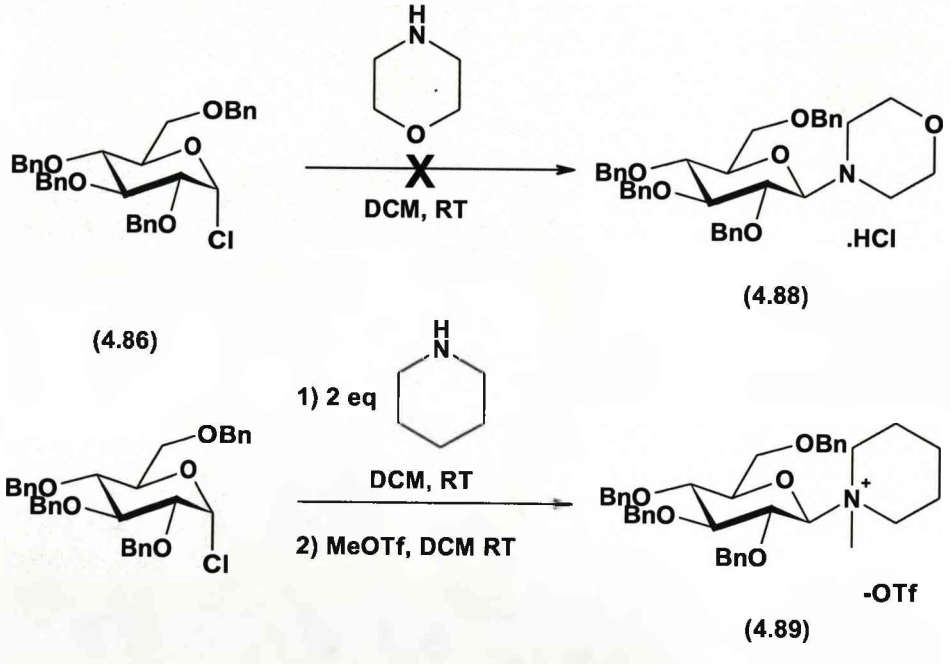


Scheme 4.7.27

NMR analysis of the crude material before reaction suggested the presence of the α-mesylate sugar **(4.87)** (~60 % by NMR integration). We decided that due to the suspected instability of the mesylate sugar, we would use it immediately without purification in the reaction with morpholine. Analysis of the reaction by NMR was difficult to interpret due to overlap of protons. By TLC there appeared to be no reaction; knowing more about the nature of the compounds this could have been due to hydrolysis of the product on silica gel. We analysed the reaction by mass spectrometry, which gave no indication of product. We therefore decided that using the more stable chloro-sugar **(4.86)** would be a better option, as this could be easily isolated and characterised.

Reaction of the chlorosugar **(4.86)** with amines, morpholine and N-methylmorpholine, again resulted in no desired product being isolated from the reactions (scheme 4.7.28). Upon column chromatography, only the chlorosugar **(4.86)** and hemiacetal **(4.85)** were isolated.

Due to the literature evidence suggesting the labile nature of **(4.88)** under acidic conditions, we thought that it was possible that product **(4.88)** was forming, but hydrolysing in the presence of hydrochloric acid generated *in situ*. We then carried out the reaction with piperidine, but used two equivalents in the hope that the second equivalent would form piperidine.HCl (scheme 4.7.28). On analysis of the reaction by NMR we suspected that the desired product had formed. We saw no evidence for elimination during the reaction. Purification by column chromatography was avoided due to the suspected labile nature of the product. The crude reaction mixture was taken and reacted with methyl triflate to form the quaternary ammonium salt **(4.89)** (scheme 4.7.28). This was then purified by column chromatography and isolated in 38 % yield.



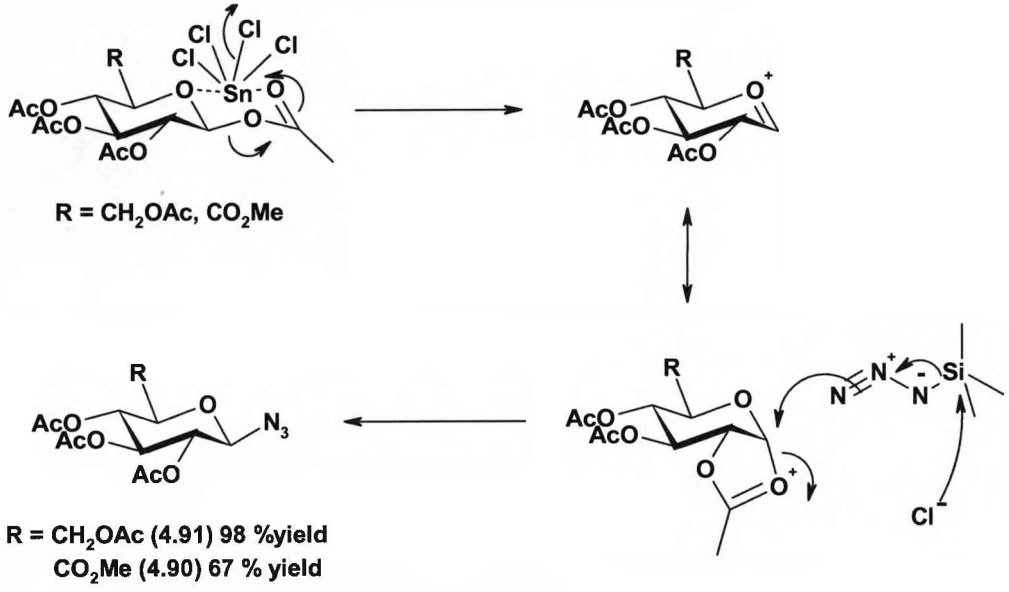
Scheme 4.7.28

We then decided to change our direction and look at introducing nitrogen to the anomeric position via azides which have been synthesised previously.

Anomeric Azides

Azides are known in both the glucuronic acid[52] and glucose series[53,54]. The azidosugar in the GA series can also be synthesised from the glucose series by oxidising the primary alcohol to the carboxylic acid using TEMPO catalysed oxidation[55].

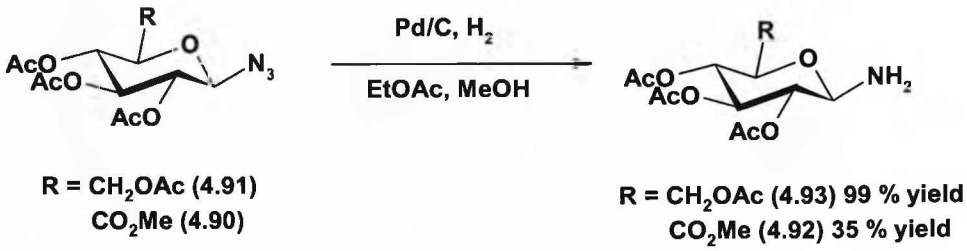
We found that using Tin (IV) chloride in catalytic amounts and azidotrimethylsilane as the azide donor, we could convert the anomeric ester to azide in both the glucuronic acid **(4.90)** and glucose series **(4.91)** (scheme 4.7.29). We also tried Titanium chloride as a milder Lewis acid but this gave no product.



Scheme 4.7.29: Mechanism in the synthesis of the β-azidosugar

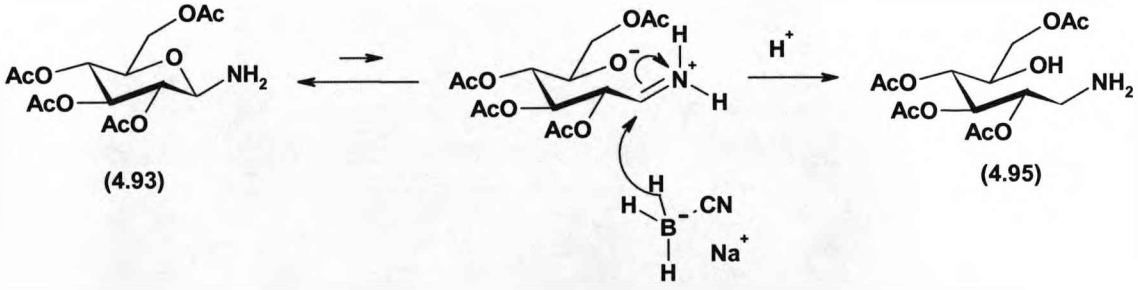
The glucose intermediate **(4.91)** can then be reduced using palladium catalysed hydrogenation[56,57,58] (scheme 4.7.30). Parekh *et al.*[59] used Pd/C, H_2 with ethanol as solvent in the reduction of galactose azide. Esteves *et al.*[60] carried out the reduction of the glycosyl azide **(4.91)** using ethyl acetate and methanol as solvent with no purification. We found that recrystallisation could be carried out using ethanol.

The azidoglucuronide **(4.90)** can also be reduced to give the aminoglucuronide **(4.92)** as has been previously reported by Pitt *et al.*[52] (scheme 4.7.30). We modified their reaction slightly by using MeOH/ethyl acetate (as with the glucose series **(4.93)**) in place of THF.



Scheme 4.7.30: Reduction conditions

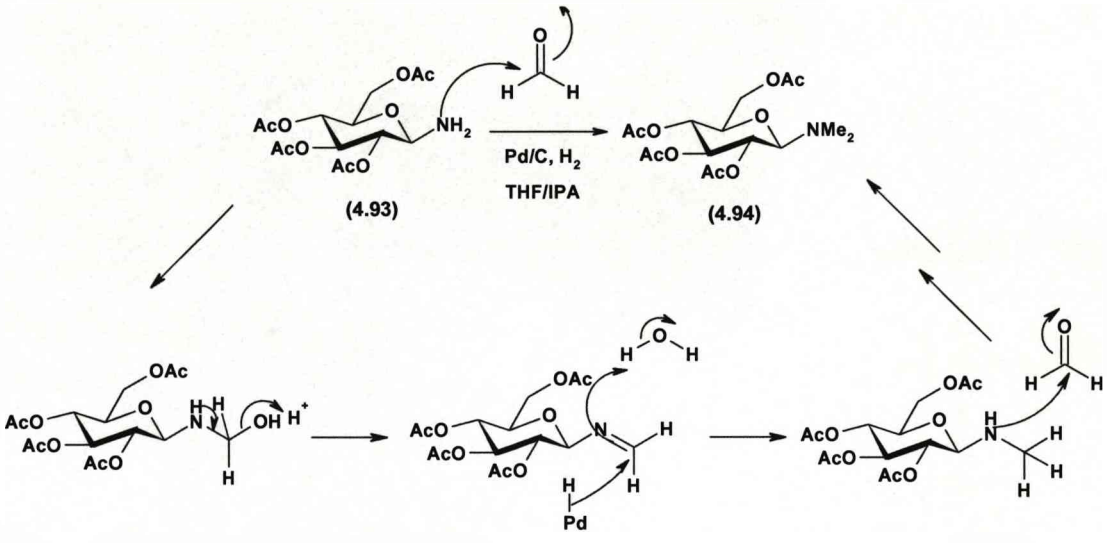
The next step in the sequence is a reductive *N*-dimethylation. Initial reactions were performed using acidic conditions such as; Eschweiller-Clarke reductive amination using paraformaldehyde and acetic acid, Pd/C hydrogenation with formic acid and paraformaldehyde, and sodium cyanoborohydride under acidic conditions in the presence of paraformaldehyde. All of these reactions gave no dimethylamino sugar **(4.94)**, only hydrolysis. We tried $NaCNBH_3$ under neutral conditions in the presence of 37 % aq formaldehyde, NMR and MS suggested that we had reduced the imine formed *in situ* (scheme 4.7.31), yielding an acyclic amine **(4.95)**.



Scheme 4.7.31: Possible mechanism of reaction with Sodium cyanoborohydride

We carried out the reductive amination under neutral conditions using, 37 % aq formaldehyde, Pd/C and H_2 to give the dimethylamino sugar **(4.94)** in the glucose

series (scheme 4.7.32). The yield was variable (40-92 %) due to hydrolysis on silica gel during purification. This reaction suggests that it was the acidic conditions in previous reactions that were not tolerated.

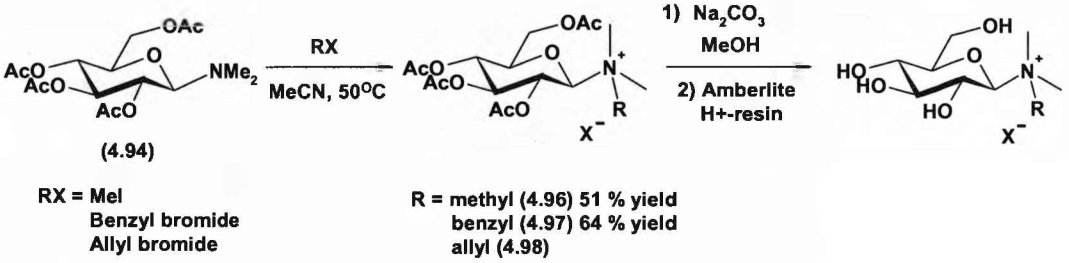


Scheme 4.7.32: Conditions used for the reductive *N*-dimethylation in the glucose series

These conditions did not work for the glucuronic acid series **(4.92)**. None of the by-products were isolated, and NMR was difficult to interpret. It could be due to the amino group being less reactive in the glucuronic acid series **(4.92)**. The methyl ester, with its electron withdrawing properties may have reduced the ability of the amino group to react with the formaldehyde.

Quaternisation Reaction of Dimethylamino Sugar to form Quaternary Ammonium Salts.

Synthesis of quaternary ammonium salts via a quaternisation reaction of **(4.94)** with electrophiles was carried out. We were able to successfully quaternise **(4.94)** with methyl iodide, benzyl bromide and allyl bromide to give **(4.96)**, **(4.97)**, and **(4.98)** respectively (scheme 4.7.33).



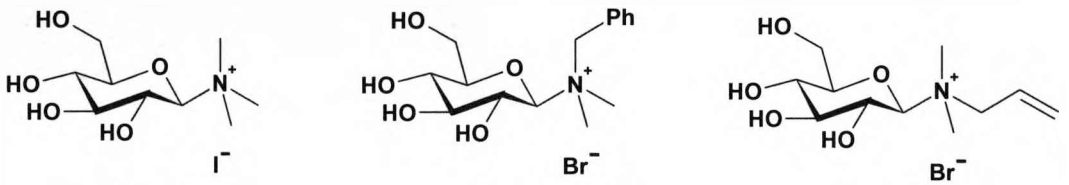
(4.94)

RX = MeI
Benzyl bromide
Allyl bromide

R = methyl (4.96) 51 % yield
benzyl (4.97) 64 % yield
allyl (4.98)

Scheme 4.7.33

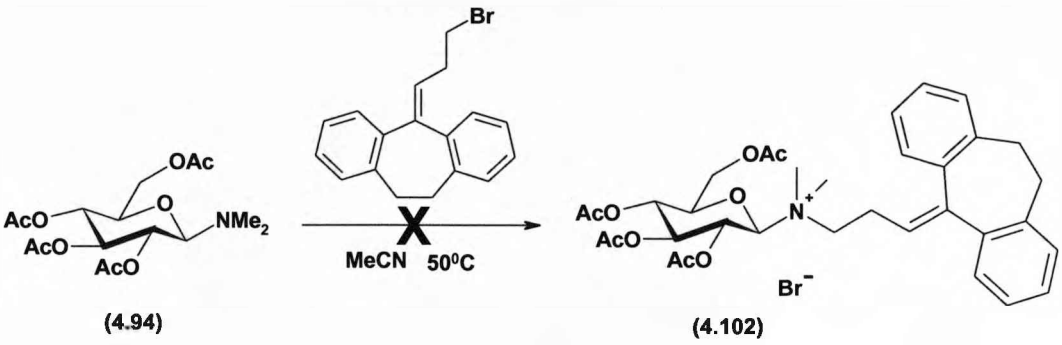
We had concerns about the stability of the products during deprotection as it has been reported that such quaternary ammonium compounds are able to form anhydro sugars in the presence of strong base[61]. The acetate protecting groups were removed using sodium carbonate in methanol (scheme 4.7.33) to give **(4.99), (4.100)** and **(4.101)** with no degradation (scheme 4.7.34). A simple workup was required for the final step; we found amberlite H^+ resin could be used to quench the reaction. The advantage of the resin was that it could be filtered off leaving just the product in solution.



(4.99) 87 % yield **(4.100) 72 % yield** **(4.101) 98 % yield**

Figure 4.7.34

This was enabling work to try to prepare Amitriptyline-N^+-Glucoside **(4.102)** and related drug like products, by quaternisation with the respective electrophile of the side chain (scheme 4.7.35). No success has been obtained with this pathway, despite trying several different leaving groups, side chain and solvents (table 4.7.36)



(4.94) (4.102)

Scheme 4.7.35: Theoretical quaternisation step to form the protected Amitriptyline N^+-Glucoside

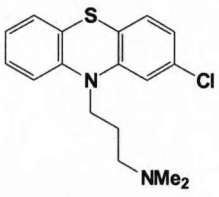
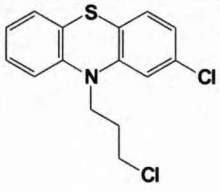
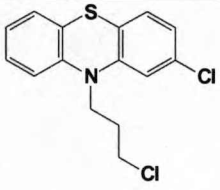
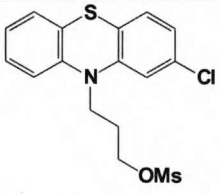
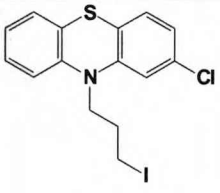
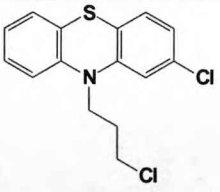
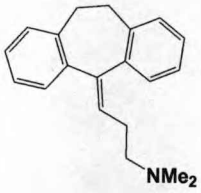
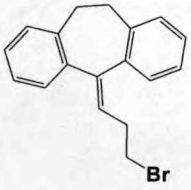
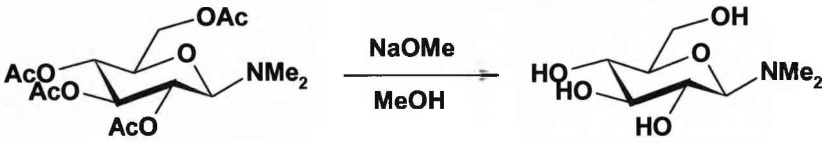
Drug	Reactant	Solvent	Conditions
Chlorpromazine  **(4.103)**	 **(4.104)**	NMP	200°C, microwave
	 **(4.104)**	MeCN	50°C
	 **(4.105)**	MeCN	50°C
	 **(4.106)**	DMF	50-80°C
	 **(4.104)**	Acetone, NaI	50°C
Amitriptyline  **(4.54)**	 **(4.107)**	MeCN	50^{0}C

Table 4.7.36: Substrates used in the quaternisation reactions

It appears that the nitrogen on the sugar is not reactive enough to form a quaternary salt with electrophiles which are less reactive than methyl iodide and benzyl bromide.

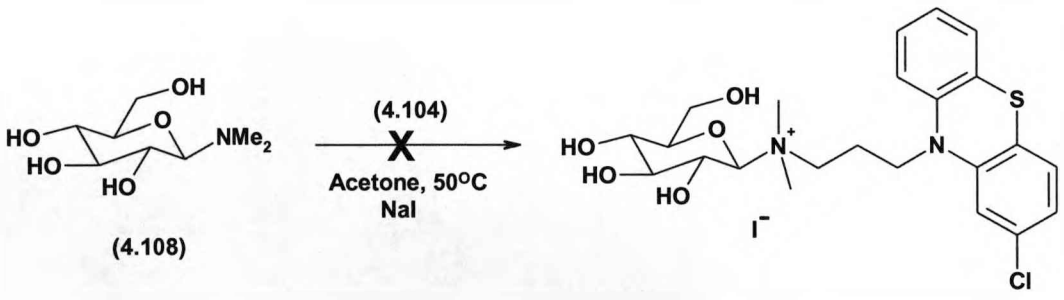
Our next step was to remove the electron withdrawing acetate protecting groups to try to improve the nucleophilicity of the nitrogen. Triethylamine in methanol was tried first, but this gave us no success. We next looked at sodium methoxide directly in methanol and this gave us the desired product **(4.108)** (scheme 4.7.37).



(4.94) **(4.108)**

Scheme 4.7.37

We made chlorpromazine **(4.103)** (table 4.7.36) a focus of the work, and we attempted to react the chloro-derivative **(4.104)** (table 4.7.36) (scheme 4.7.38). A Finkelstein reaction[62] was used to generate the iodo *in situ*, as there were concerns on the stability of the iodo-derivative **(4.106)** (table 4.7.36) from previous reactions.

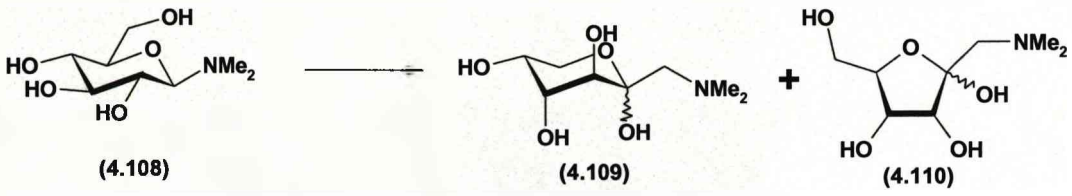


(4.108)

Scheme 4.7.38: Conditions for the *in situ* Finkelstein reaction and quaternisation reaction

Again this reaction gave us no quaternary ammonium salt. The chlorpromazine derivative appeared to decompose under these conditions. There were also concerns about the stability of **(4.108)**. The free hydroxy groups potentially mean

that the Amadori rearrangement could occur (scheme 4.7.39). It is difficult to determine if the Amadori rearrangement had occurred due to the complicated NMR spectrum.



Scheme 4.7.39: Potential Amadori rearranged products

We decided that this route was not worth pursuing and moved on to look at the reaction of free sugars with primary and secondary amines.

Crystal Structure Data, and its relation to the Anomeric Effect

As discussed in the introduction (p 26), the anomeric effect is a well accepted phenomenon. On the other hand, the reverse anomeric effect is very much debated and its existence had been questioned.

During the synthesis of the trimethyl ammonium glucoside, we were able to crystallise both the protected **(4.96)** and deprotected **(4.99)** products, giving us information on the bond lengths of the two species (figure 4.7.40). The anomeric C-N^+ bond length found in **(4.96)** is 1.523 Å and the deprotected species **(4.99)** has a bond length of 1.522 Å which are the same giving experimental error (± 0.005 Å). These bond lengths are consistent with those found in a trimethyl ammonium group attached to a secondary carbon, with the substructure Me_3N^+-CHR_2 (R = alkyl), where the N^+-C bond is found to be 1.537 Å (± 0.008 Å).

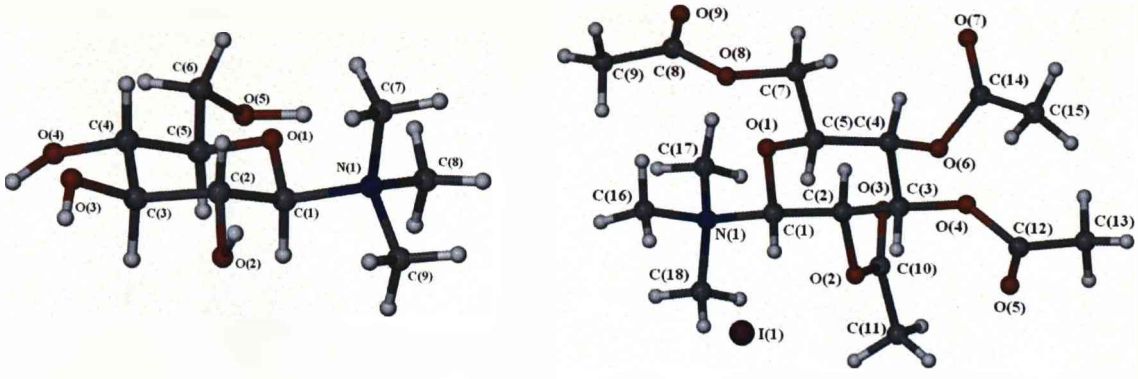


Figure 4.4.40: Crystal structures of **(4.99)** and **(4.96)**

The Me_3N^+ group can only act as an σ-acceptor, so that n_O-$\sigma^*_{C\text{-}O}$ donation can only affect the exocyclic bond. It is believed that sterically it is more favourable for such quaternary ammonium salts to adopt the β-configuration, and that this effect is predominant over the anomeric effect.

Ideally a crystal structure of the α-trimethylammonium glucoside (figure 4.7.41) could give insight as to whether such compounds behave as expected, adhering to the anomeric effect. If we saw a lengthening of the exocyclic C-N^+CH_3 bond, this would suggest that the anomeric effect exists with such compounds, and would give more evidence to the fact that the reverse anomeric effect does not exist.

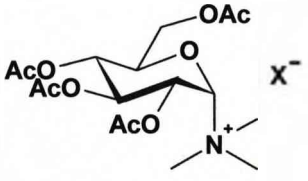


Figure 4.7.41

4.7 Results and Discussions Part II: the Synthesis of N^+-Glucuronides and Glucosides

The Reaction of Secondary Amines with Glucose

It is known that a direct reaction of glucose with piperidine will give *N*-Glucosylpiperidine **(4.111)**[20,63]. We have confirmed this reaction, and conducted further investigations with other cyclic secondary amines; morpholine, N-methylpiperazine and pyrrolidine to give **(4.112), (4.113)** and **(4.114)** respectively (figure 4.9.1). There are many drugs that contain morpholine, piperidine, piperazine and pyrrolidine structural elements, and all are potential candidates for *N*-glucuronidation.

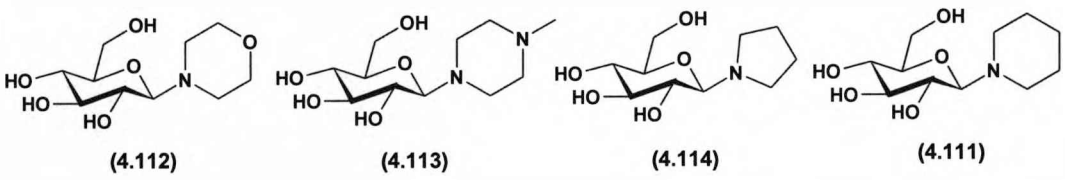
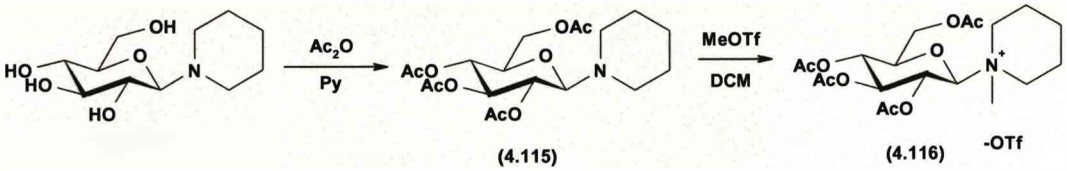


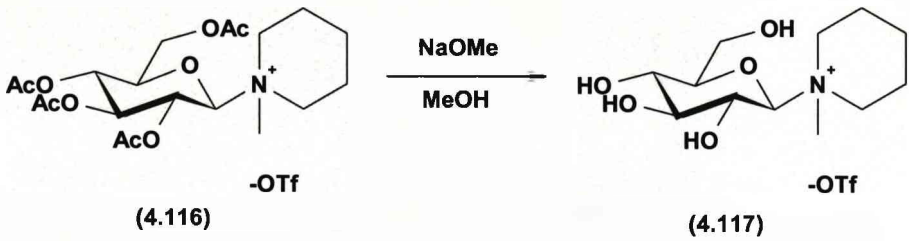
Figure 4.9.1: Cyclic amine glycosides

An acetylation of the free hydroxyl groups was carried out on amines **(4.111), (4.112)** and **(4.113)** using acetic anhydride and pyridine as solvent. An attempted quaternisation reaction was carried out on the piperidine derivative **(4.115)** using methyl iodide. We found that this methyl iodide did not react with the intermediate **(4.115)**. We then looked at using methyl triflate, and were able to successfully quaternise **(4.115)** (scheme 4.9.2). We then tried to quaternise the morpholine derivative **(4.116)**, but found that methyl triflate did not react, showing the less reactive nature of the morpholine nitrogen.



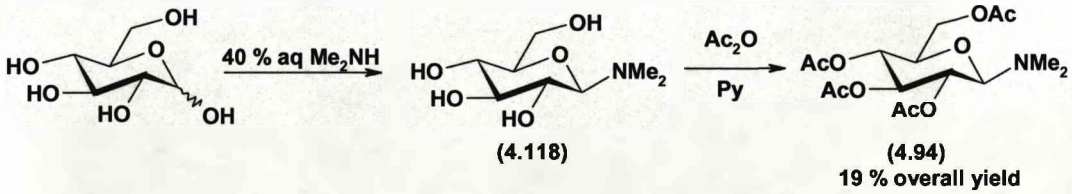
Scheme 4.9.2

A deprotection was then carried out on **(4.116)** using sodium methoxide (scheme 4.9.3) to give **(4.117)**. We found that using our previous method of Na_2CO_3 and methanol gave no deprotection



Scheme 4.9.3

We also looked at the reaction of glucose with aqueous dimethylamine (scheme 4.9.4). Once we had carried out the condensation reaction, NMR analysis showed the presence of some remaining glucose together with the β-anomeric product **(4.118)**. We then acetylated any free hydroxyl groups using acetic anhydride and pyridine (scheme 4.9.4). This gave us the dimethylamino sugar **(4.94)** in 19 % yield, compared to the earlier azide route which gives a yield of 39 % over three steps.

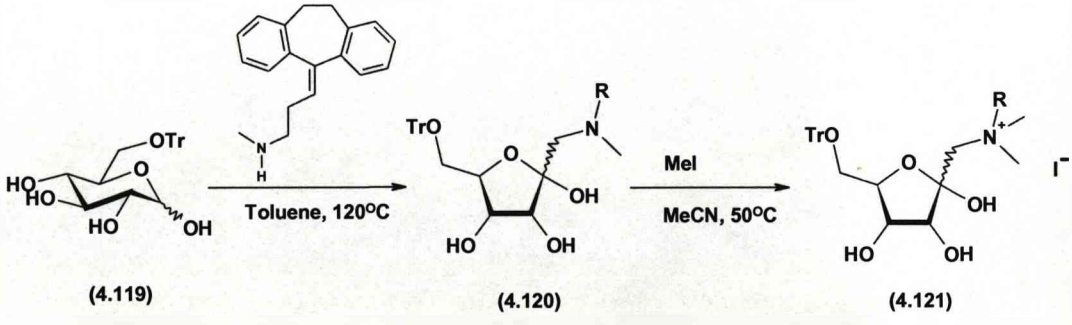


Scheme 4.9.4: Reaction conditions to form **(4.94)** in two steps

Secondary Amine, Nortriptyline in the Reaction with the Hemiacetal 6-O-Trityl Glucose

We needed an organic soluble source of glucose to use in our reactions with more lipophilic secondary amines. We chose 6-*O*-trityl glucose **(4.119)** as a stable crystalline compound which is easy to prepare from glucose and trityl chloride in the presence of pyridine.

Initially we took trityl glucose **(4.119)** and Nortriptyline **(4.64)** and heated them together at 120°C in toluene. NMR analysis suggested that we had formed the Amadori rearranged product **(4.120)** (scheme 4.9.5). The pyranose ring characteristics were no longer present; there appears to be a mixture of isomers, giving rise to two sets of triplets at 5.7-5.8 ppm for the nortriptyline olefenic proton of each isomer. The spectrum also shows two singlets at 2.32 ppm and 2.33 ppm accounting for the NCH_3 protons. In the desired product we see the NCH_3 signal further downfield at 2.4 ppm. The NMR is complex due to the nature of nortriptyline **(4.64)** giving rise to broad signals, but all of these characteristics led us to believe the presence of **(4.120)**.



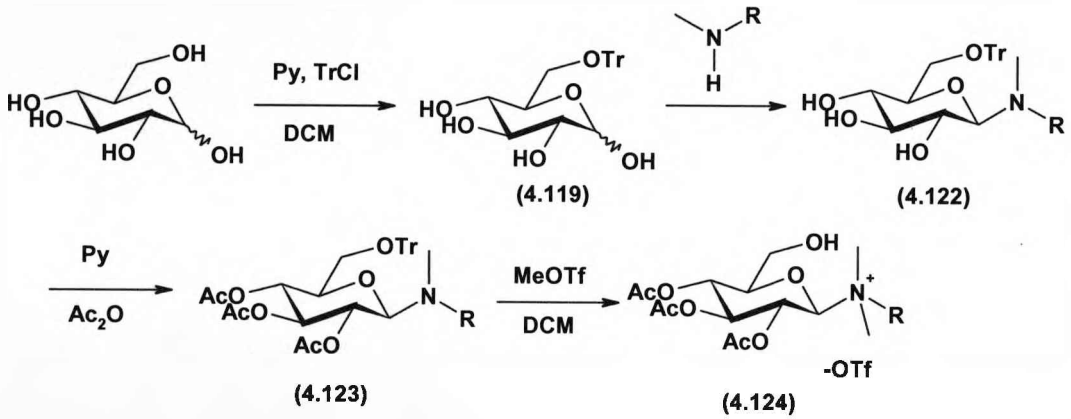
(4.119) (4.120) (4.121)

Scheme 4.9.5: Amadori rearranged product **(4.120)** formed at 120°C and product from MeI quaternisation **(4.121)**

Further confirmation that the rearranged species had formed was that the quaternisation reaction used methyl iodide which contained traces of acid and water. The desired product hydrolyses in the presence of acid, but the Amadori

rearranged product **(4.120)** is able to react forming the quaternary ammonium salt **(4.121)**.

Under milder conditions using DCM as solvent at room temperature, Nortriptyline **(4.64)** undergoes the desired condensation reaction with 6-*O*-trityl glucose **(4.119)** to give pure β-anomer (scheme 4.9.6). Column chromatography was used to purify this intermediate **(4.122)**, but again we found significant hydrolysis occurred on the silica gel which lowered our yields to 49 % (from crude NMR analysis yield ~80 %). Quaternisation of **(4.122)** was not possible due to reaction of the 2,3,4-OH groups with methyl triflate. We then protected the hydroxy groups by acetylation to give **(4.123)** in 79 % yield, and were then able to quaternise **(4.123)** with methyl triflate. During this step we saw removal of the trityl group that we presume is via trace acid hydrolysis. Purification of **(4.124)** was carried out by rapid column chromatography, hydrolysis of the aglycone occurred during the process lowering our yields to 59 %.

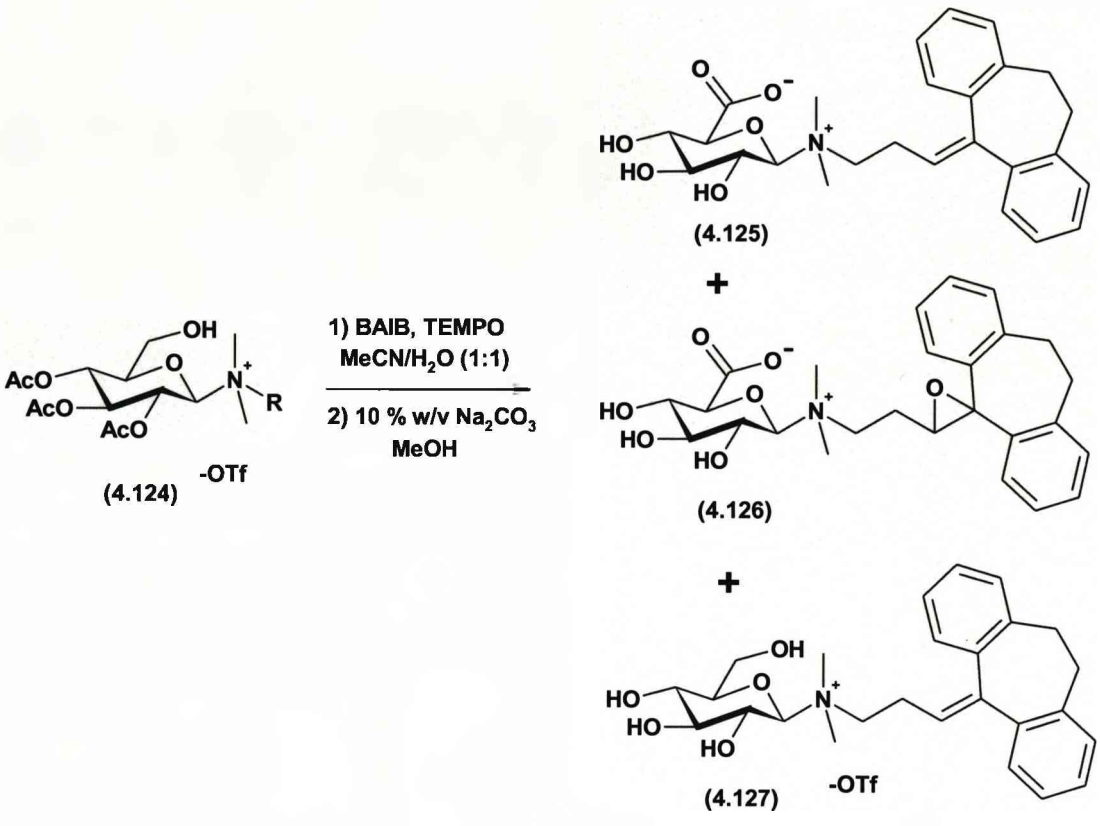


CH_3NHR = Nortriptyline (4.64)

Scheme 4.9.6: Reaction Conditions to form the quaternary ammonium salt **(4.124)**

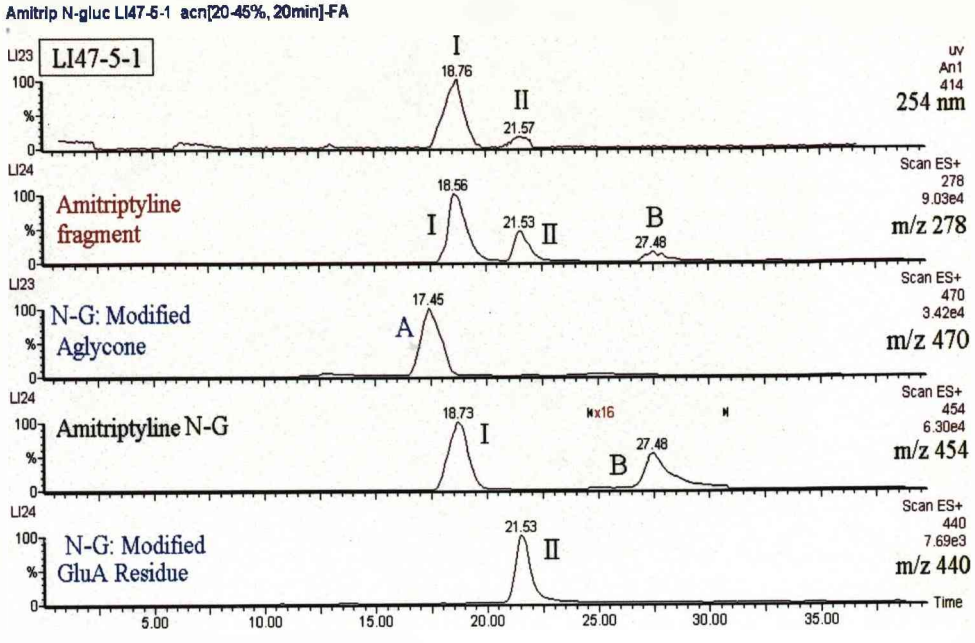
Having removed the 6-*O*-trityl group, oxidation of the primary alcohol to the carboxylic acid would take us through to the glucuronic acid series **(4.125)** (scheme 4.9.7). We looked at various conditions for this step and eventually found that BAIB, and TEMPO in $MeCN/H_2O$ gave us the desired product with some over-oxidation of the amitriptyline portion **(4.126)** (scheme 4.9.7). Epp *et al.* used similar conditions to oxidise 5'-hydroxymethylene of nucleosides to 5'-carboxylates[64].

Previous attempts had involved the use of TEMPO and harsh oxidising reagents e.g. NaOCl, under basic conditions which we believe caused hydrolysis of the aglycone. We were limited in the oxidants we could use due to the double bond present in the aglycone **(4.54)**.



Scheme 4.9.7: Oxidation then hydrolysis of **(4.124)** giving the desired product **(4.125)** plus by-products **(4.126)** and **(4.127)**

We were able to analyse the BAIB, TEMPO reaction by LCMS carried out by James Maggs from the Department of Pharmacology. He found that the reaction mixture contained both Amitriptyline N^+-Glucoside **(4.127)** (peak II *m/z* 440 LCMS 4.9.8) and Amitriptyline N^+-Glucuronide **(4.125)** (peak I *m/z* 454 LCMS 4.9.8) (scheme 4.9.7). There was evidence for the presence of a by-product due to over oxidation, presumably epoxidation of the double bond as well as oxidation of the primary alcohol **(4.126)** (peak A *m/z* 470 LCMS 4.9.8) (scheme 4.9.7).



LCMS 4.9.8

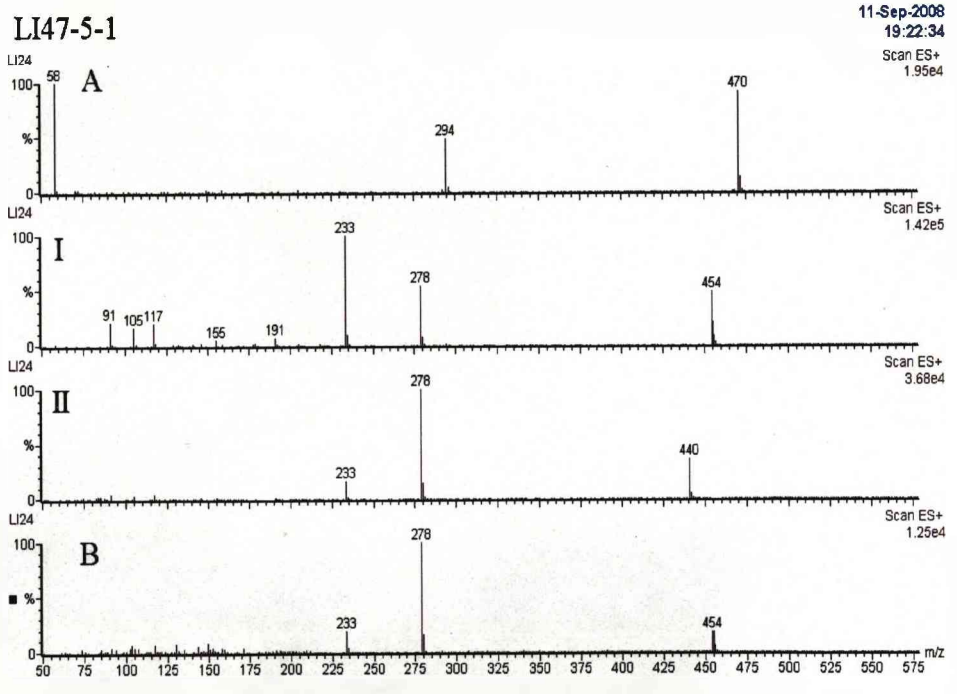


Figure 4.9.8: LCMS trace and respective MS data collected by James Maggs of The University of Liverpool. Peak A, **(4.126)**. Peak I, **(4.125).** Peak II, **(4.127)**.

Summary

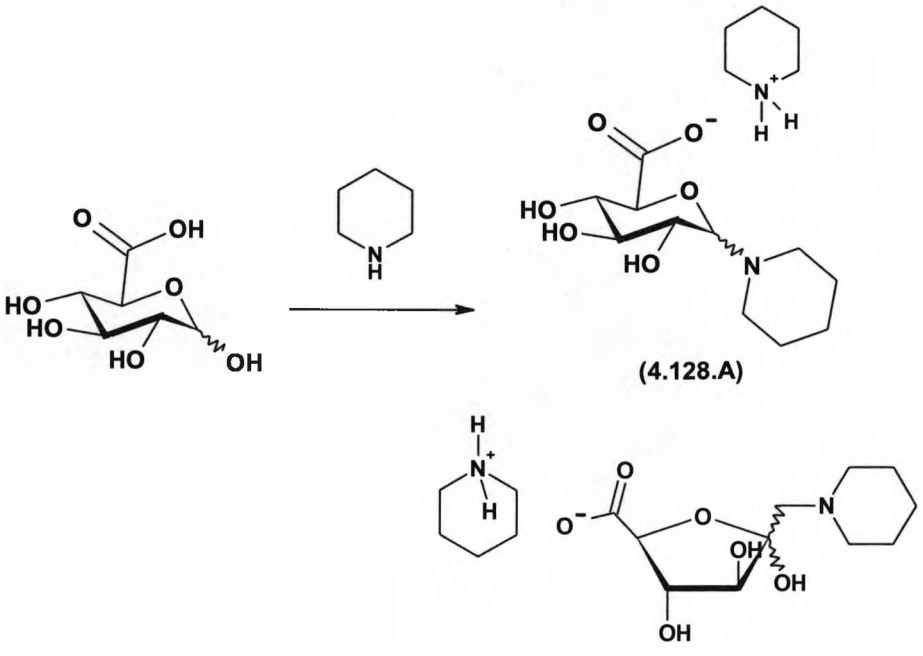
We wanted to fully evaluate the condensation reaction in the glucose series, as we believed that the glucose series would be more reactive than the GA series during these reactions. This route gave us access to higher yields (scheme 4.9.6) (20 % over four steps) of quaternary ammonium salts of drug examples, which up until this point had not been achieved successfully using the nucleophilic substitution route (scheme 4.7.10 and scheme 4.7.12) (0.04 % over two steps). We wanted to find an oxidation method that would be compatible with Amitriptyline **(4.54)**, but unfortunately we were not successful. This method would be suitable for a quaternary ammonium salt which contains no functional groups capable of being oxidised under the conditions used. It is still a viable means for the synthesis of N^+-Glucuronides.

The Reaction of Secondary Amines with Glucuronic Acid

The reaction of aliphatic primary amines and the sodium salt of glucuronic acid is known.[19] Takitani *et al.*[19] used the sodium salt of glucuronic acid in preference to glucuronic acid itself as they found that not only did the amine react at the anomeric centre, but it also formed a salt with the carboxylic acid moiety.

We reacted piperidine and glucuronic acid and obtained the desired β-product **(4.128.A)**. Measured by NMR intergration and elemental analysis it appeared that piperidine had undergone the condensation reaction and had also formed the salt at the carboxylic acid. By NMR analysis it appeared that there was another product which we suspected to be due to Amadori rearrangement **(4.128.B)**. The glucose equivalent gives only the β-*N*-glycosyl piperidine **(4.111)** (scheme 4.9.1). The NMR spectrum shows an integration of 50:50 β-product and suspected rearranged product. The second product does not show the usual coupling pattern that a pyranose sugar generally gives, but we know that it also contains two piperidine molecules, and gives the same elemental analysis as the β-product.

Reaction of glucuronic acid with morpholine and pyrrolidine proved to be very exothermic; NMR suggested that possible side reactions had occured. On standing for 16 hours in DMSO, the NMR spectrum showed an increase in the suspected rearranged product **(4.128.B)**. The formation of the rearranged product is probably due to acid catalysation, by glucuronic acid.

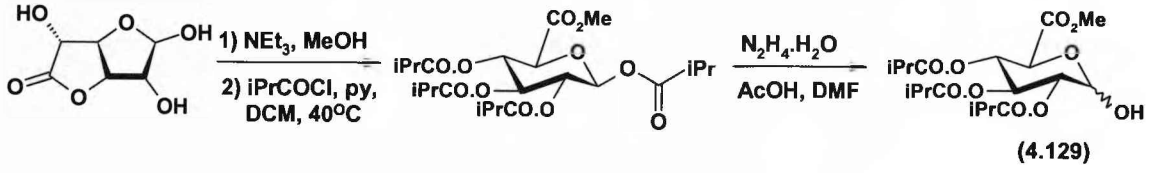


Scheme 4.9.9: Products formed during reaction of piperidine and Glucuronic acid

Secondary Amines in the Reaction with the Hemiacetal in the GA Series

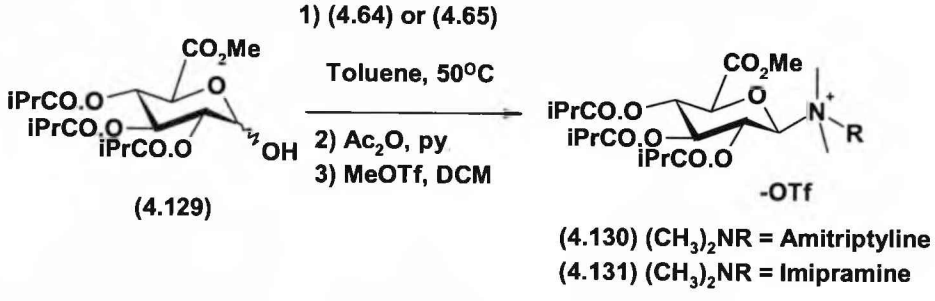
To avoid having to carry out a separate oxidation step we show in this section that the same condensation reaction carried out with 6-*O*-trityl glucose **(4.119)** and Nortriptyline **(4.64)** can be applied to the glucuronic acid series **(4.129)** (scheme 4.9.10).

The isobutyrate protected hemiacetal **(4.129)** is used as the sugar source. Starting from the commercially available glucurono-3,6-lactone the hemiacetal **(4.129)** can be synthesised in two steps with an overall yield of 45 % (scheme 4.9.10). It is a more stable intermediate than anomeric sugar halides and can be stored in a desiccator for at least 6 months without any decomposition. We chose the isobutyrate protecting groups as a result of our earlier investigations. When using the acetate protected bromosugar **(1.6)**, we found that acetylation of the amine occurred (scheme 4.7.11, p. 107). We saw no evidence of the secondary amines used reacting with the methyl ester to form the amide.



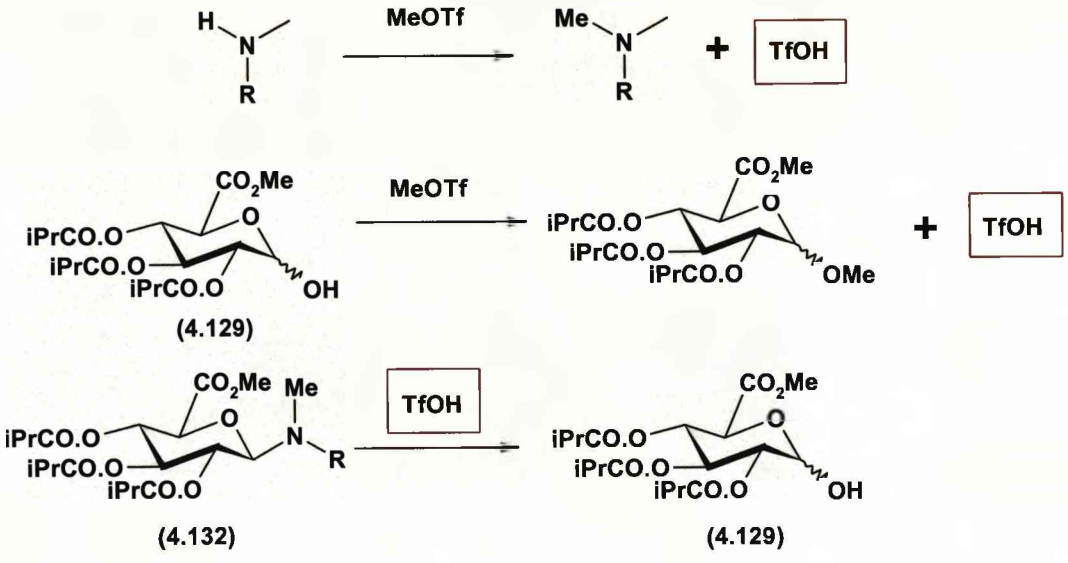
Scheme 4.9.10

Nortriptyline **(4.64)** and Desipramine **(4.65)** react readily with **(4.129)** (scheme 4.9.11). This method gives selectively the β-anomer, thought to be due to steric effects.



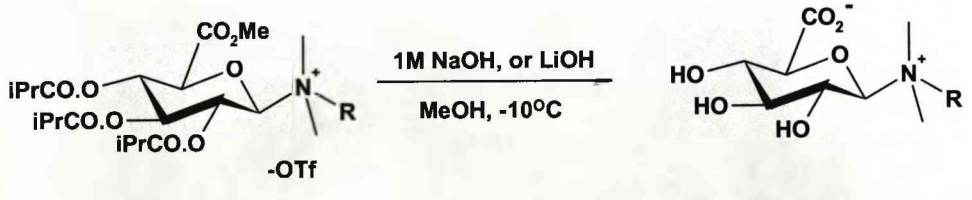
Scheme 4.9.11

We then carry out an acetylation reaction of any remaining amine **(4.64 or 4.65)** and hemiacetal **(4.129)**. Reaction of these two species with methyl triflate in the next step would generate triflic acid, which could potentially cause hydrolysis of the intermediate **(4.132)** (scheme 4.9.12).



Scheme 4.9.12: The side reactions that could occur in the presence of triflic acid

The quaternisation with methyl triflate is then carried out under the same conditions used in the glucose series (scheme 4.9.11). We were able to isolate the intermediates **(4.130)** and **(4.131)** (scheme 4.9.11) by filtering the crude reaction mixture through a pad of silica gel. This was carried out efficiently to avoid hydrolysis of the aglycone. At this stage the intermediates had a purity of ~80 %. The next step was to remove the *O*-isobutyryl and methyl ester protecting groups, this is achieved using 1M NaOH or 1M LiOH in MeOH to give **(4.133)** and **(4.134)** (scheme 4.9.13) *(see later for a full discussion on the deprotection step).*



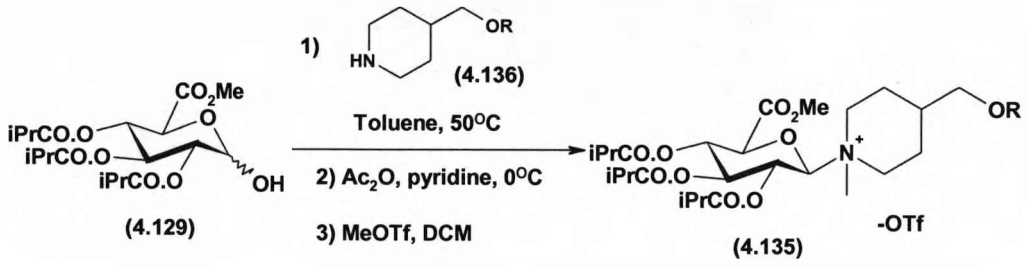
$(CH_3)_2NR$ = Amitriptyline (4.130)
Imipramine (4.131)

$(CH_3)_2NR$ = Amitriptyline (4.133)
Imipramine (4.134)

Scheme 4.9.13

Formation of an N^+-Glucuronide currently being investigated by AstraZeneca

We looked at forming the N^+-Glucuronide of a drug currently being investigated by AstraZeneca. The suspected N^+-Glucuronide formation is via the *N*-methylpiperidine **(4.135)** (scheme 4.4.14). Our strategy to form the N^+-Glucuronide was to react the hemiacetal with the des-methyl derivative **(4.136)**, then carry out the acetylation and quaternisation as previously done (scheme 4.9.14).



Scheme 4.9.14

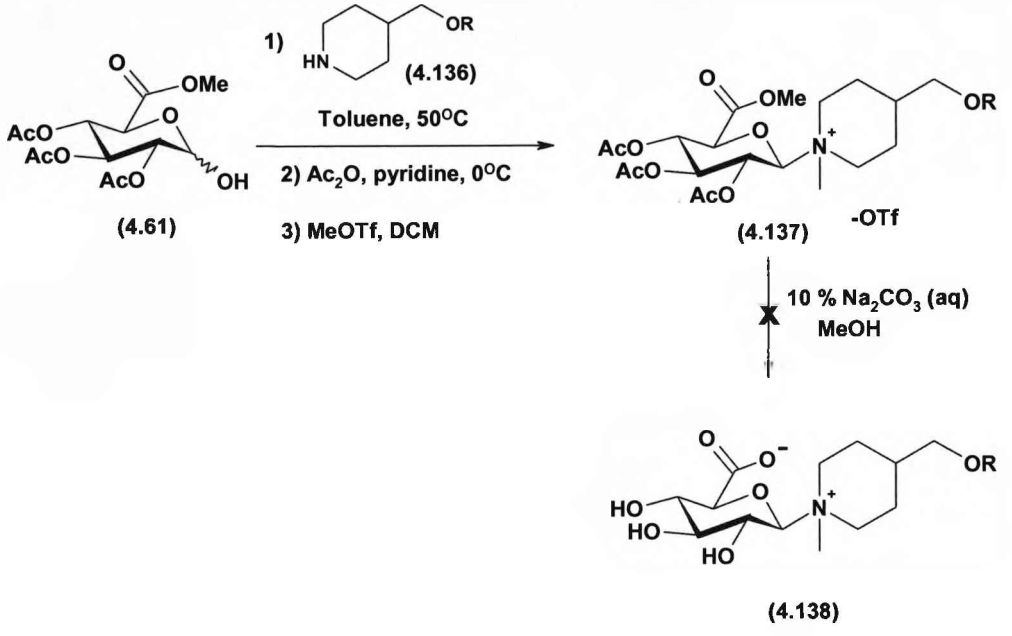
We were able to form the intermediate **(4.135)** in 23 % yield. We then tried to deprotect the isobutyrate groups to form the final product. The conditions we tried are summarised in table 4.9.15. Unfortunately we were unable to remove the isobutyrate protecting groups, the reason for this is unclear.

Base	***Equivalents***	***Solvent***
10 % w/v Na_2CO_3 (aq)	4	MeOH
1M NaOH	4	MeOH
1M NaOH	8	MeOH

Table 4.9.15

To overcome the problem we changed the *O*-isobutyryl groups to *O*-acetyl protecting groups **(4.61)** (scheme 4.9.16). We had avoided using acetate groups due to previous investigations where we saw significant acetylation of Desipramine (scheme 4.7.11).

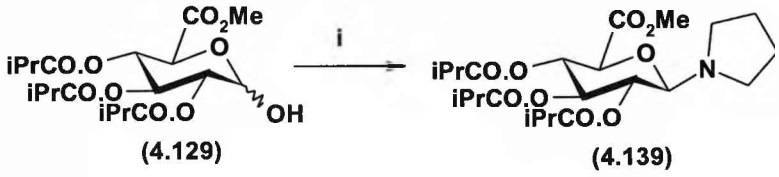
The condensation reaction was carried out and there was no evidence for acetylation of the amine. The protection of unreacted amine **(4.136)** and hemiacetal **(4.61)** was carried out by acetylation as in previous examples. Quaternisation of the piperidine nitrogen was done in the usual way with methyl triflate to give **(4.137)** (14 % yield) (scheme 4.9.16). The deprotection was unsuccessful in this series, using 10 % w/v Na_2CO_3 (aq) in MeOH (scheme 4.9.14). We isolated from the reaction glucuronic acid and the parent drug, showing hydrolysis of the glycosidic bond during deprotection.



Scheme 4.9.16

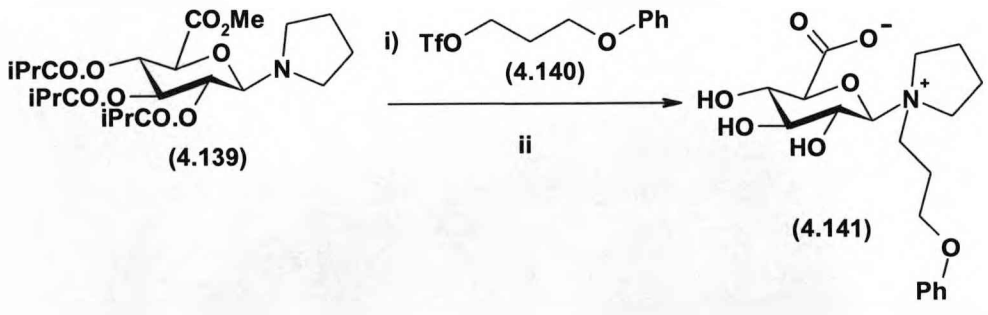
***N*-substituted Pyrrolidine N^+-Glucuronide**

We found that **(4.129)** reacts well with cyclic secondary amines such as pyrrolidine to give **(4.139)** (scheme 4.9.17). This is not surprising since it is known that secondary amines (e.g. piperidine) and primary amines react with glucose and glucuronic acid respectively to form the *N*-Glucosides/*N*-Glucuronides. NMR analysis of this reaction shows 100 % yield of the product **(4.139)**, and requires no further purification and no acetylation.



Scheme 4.9.17: i) Pyrrolidine 1.4eq, Toluene, 50°C, 100 % yield

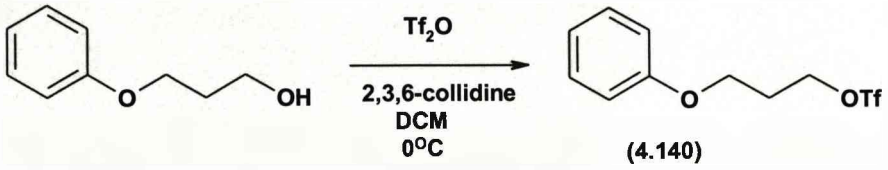
We looked at quaternisation with **(4.140)**, to show that the quaternisation step would work for electrophiles other than methyl. Reaction of **(4.139)** with **(4.140)** gave us the desired quaternary ammonium N^+-glucuronide (scheme 4.9.18). This was then deprotected as a crude mixture using 10 % w/v Na_2CO_3(aq) giving **(4.141)** in a yield of 11 % after preparative HPLC (scheme 4.9.18).



Scheme 4.9.18: i) DCE, 50°C ii) 10 % w/v Na_2CO_3 (4 eq), MeOH, -10°C 11 % yield

Formation of **(4.140)** was achieved by taking the primary alcohol and reacting this with 2,3,6-collidine and trifluoromethanesulfonic anhydride at 0°C in DCM (scheme

4.9.19). Due to high solubility of 2,3,6-collidine.HCl in DCM we used diethyl ether during extraction.

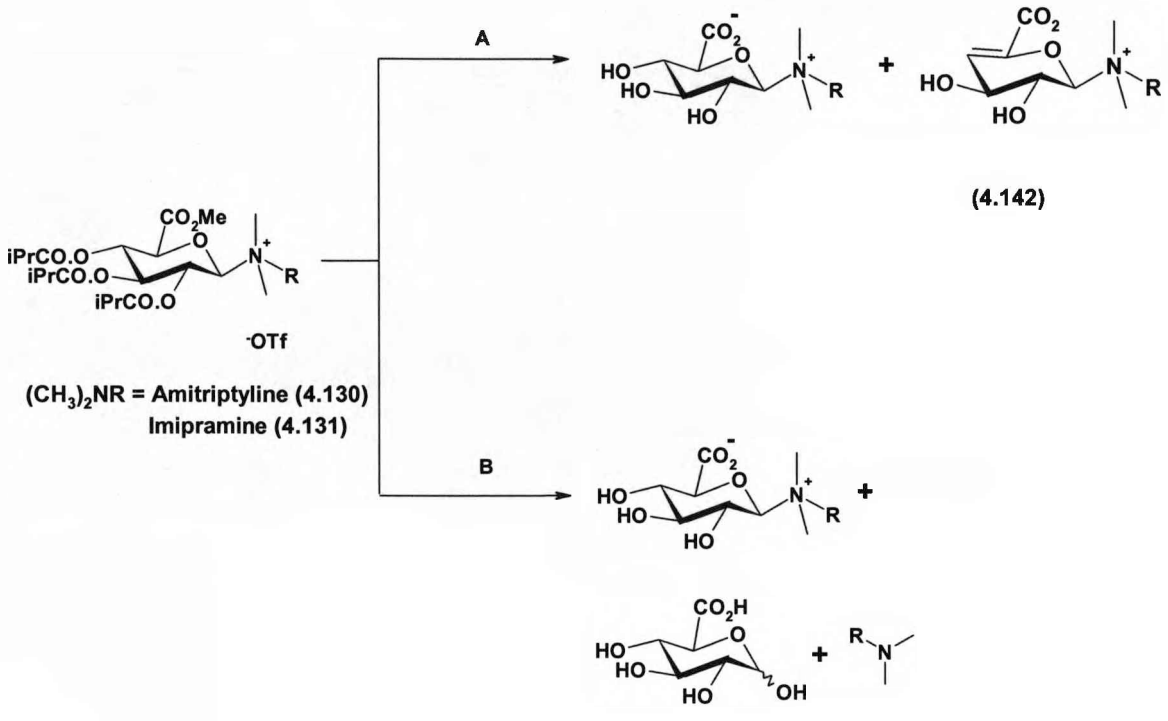


Scheme 4.9.19

Primary triflate **(4.140)** was used for optimisation studies for more complex compounds that we wished to look at later. We knew that the compounds had limited solubility, so we needed to avoid using 2,3,6-collidine that forms a salt soluble in DCM. We looked at alternatives; pyridine gave some product but in lower yield (27 % cf 37 %), due to the reaction of pyridine with the formed primary triflate. Another base used was 2,6-lutidine, but surprisingly this gave us very low yields (8 %).

Discussion of the Deprotection Step

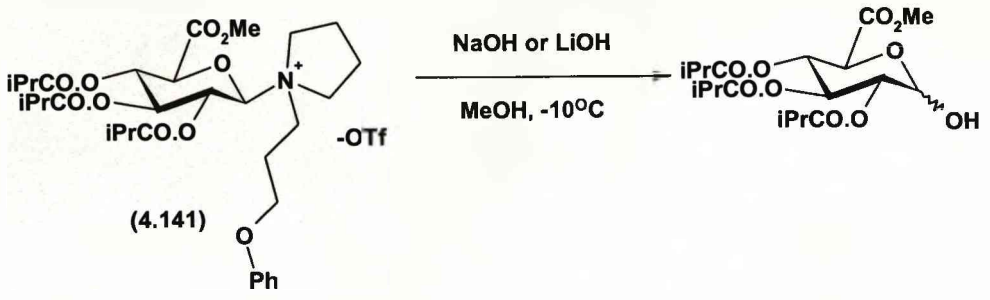
The final step in the sequence is the deprotection of the methyl and isobutyrate esters to give the zwitterionic N^+-glucuronide. The conditions used are dependent on the stability of the aglycone under basic conditions. During base hydrolysis to remove the isobutyrate esters two by-products can form; the aglycone can be liberated (pathway B scheme 4.9.20) or elimination of the 4-isobutyrate can result (pathway A scheme 4.9.20).



Scheme 4.9.20: A: 10 % w/v Na_2CO_3, MeOH, B: 1M LiOH or NaOH, MeOH -10°C

We found that the conditions to minimise both by-products were dependent on the aglycone. For both **(4.130)** and **(4.131)** either 1M NaOH or 1M LiOH were used. Although we observed hydrolysis yielding the aglycone (~30 % hydrolysis) (pathway B, scheme 4.9.20), the elimination of the 4-isobutyrate was not observed during the course of the reaction. When we used 10 % w/v Na_2CO_3; we found that the major by-product was from elimination of the 4-isobutyrate **(4.142)** accounting for ~30 % of the reaction mixture (pathway A scheme 4.9.20). This proved more difficult to remove from the final product.

The 1M NaOH or 1M LiOH conditions could not be used for **(4.141),** due to the enhanced lability of the glycosidic bond (scheme 4.9.21). Using such bases gave up to 80 % hydrolysis of the quaternary ammonium in preference to the protecting group ester hydrolysis.



Scheme 4.9.21

The optimal conditions for deprotection of **(4.141)** were found when using 10 % w/v Na_2CO_3 and methanol as solvent. The by-product formed from elimination of the 4-isobutyrate was **(4.143)** (~30 %) (figure 4.9.22), but hydrolysis of the glycosidic bond was completely avoided.

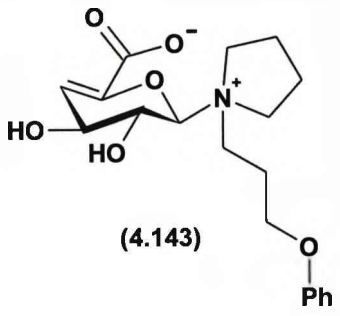
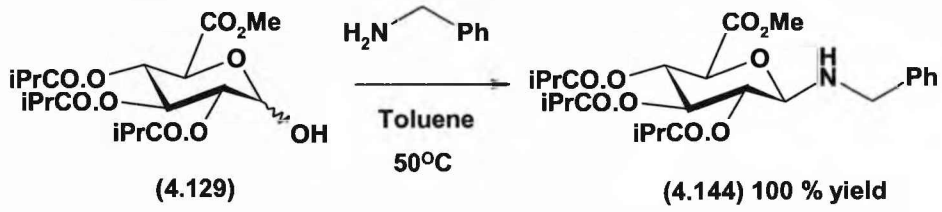


Figure 4.9.22: By-product formed during isobutyrate deprotection with 1M NaOH

The Reaction of Primary amines with the Hemiacetal in the GA Series

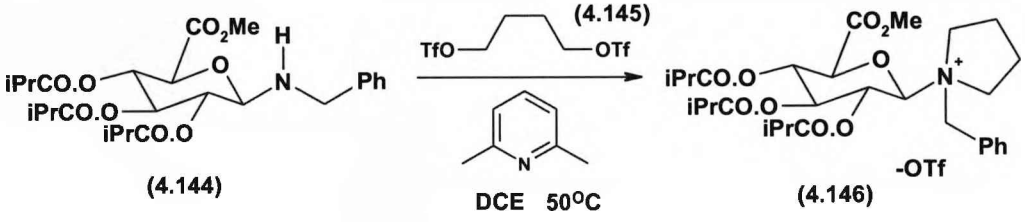
We also wanted to look at approaches that could form the pyrrolidine ring in an intramolecular fashion. To optimise this reaction we took benzylamine and the protected hemiacetal **(4.129)** under the same conditions as previously investigated (scheme 4.9.23). This reaction gave us the desired product **(4.144)** in 100 % yield, measured by NMR analysis.



Scheme 4.9.23: Reaction conditions for the condensation between benzylamine and the hemiacetal.

We then wanted to bis-alkylate the nitrogen with 1,4-butane ditriflate **(4.145)**, involving an intramolecular quaternisation.

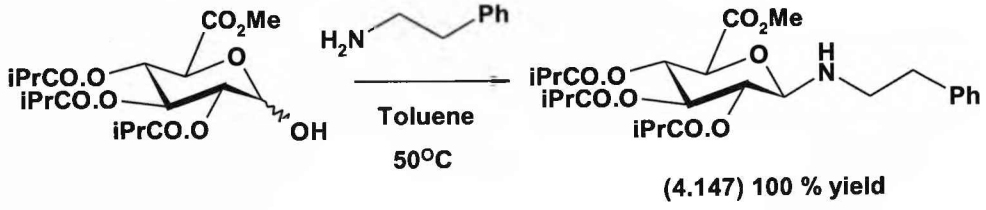
Beard *et al.*[65] have previously synthesised 1,4-butaneditriflate from THF and trifluoromethanesulfonic anhydride. We were able to isolate 1,4-butane ditriflate as a crystalline solid and use it in the proposed reaction (scheme 4.9.24).

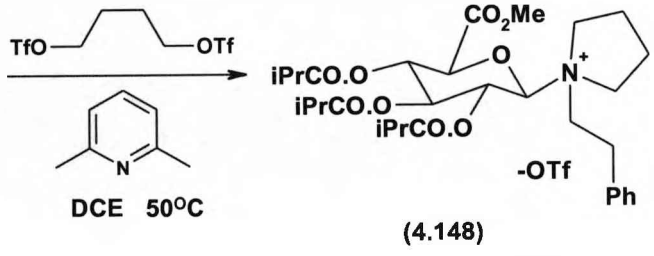


Scheme 4.9.24: Reaction conditions for the alkylation followed by intramolecular quaternisation

We carried out this reaction in the presence of 2,6-lutidine to neutralise the triflic acid generated *in situ* from the initial alkylation reaction. 2,6-Lutidine was chosen as it is a hindered, non-nucleophilic base and would therefore avoid any possible reaction with the 1,4-butaneditriflate **(4.145)**. The reaction was heated to 50°C for 24hrs. After removal of the solvent the residue was filtered through a pad of silica which was eluted with 100 % ethyl acetate, followed by 10 % Ethanol/DCM with the intermediate **(4.146)** eluting in the latter. NMR showed product present with some impurities (~ 20 %) remaining at this stage. Further purification was carried out using column chromatography, but the intermediate **(4.146)** hydrolysed during the process showing the labile and unstable nature of this compound.

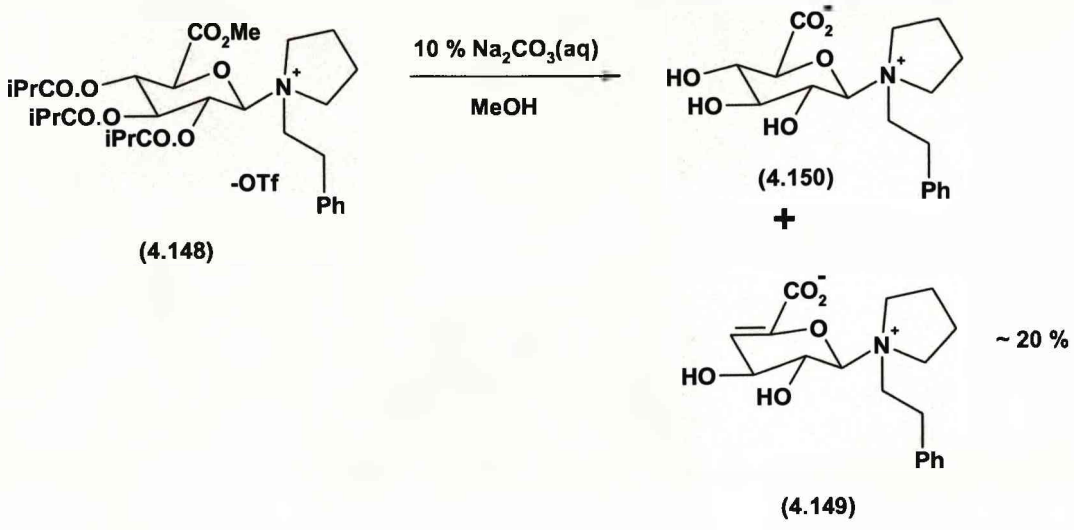
We then looked at replacing benzylamine with 2-phenylethylamine, we hoped that this would be more reactive. The same conditions for the condensation reaction were used to form **(4.147)** (scheme 4.9.25). We then carried out the alkylation and intramolecular quaternisation under the same conditions as with **(4.144).** The intermediate **(4.148)** was filtered through a pad of silica gel, at this stage the compound was ~ 70 % pure. The intermediate did not appear to be very stable so it was decided that deprotection would be carried out with no further purification.





Scheme 4.9.25

Deprotection of the isobutyryl and methyl ester groups was achieved using 10 % w/v Na_2CO_3(aq) (scheme 4.9.26). It was thought that the compound would be similar to the previous pyrrolidinium compound **(4.141)** with respect to lability. Analysis of the crude reaction mixture by NMR showed the presence of the eliminated by-product **(4.149)** (~20 %). The compound was purified by preparative TLC to give the product **(4.150)** in 10 % yield with purity of ~80 %.



Scheme 4.9.26

4.8 Conclusions and future work

During our initial investigations we looked at various sugars activated at the anomeric position to directly quaternise the tertiary amine, Amitriptyline. We found that the substitution reaction did not proceed, but the E2 reaction was significant. Despite trying to drive the reaction to the substitution product we were unable to form the desired quaternary ammonium species. The use of secondary amines appeared more promising, but poor yields ensued.

We then looked at the formation of quaternary ammonium glucosides. We hoped to optimise our route, and potentially oxidise the glucose derivative to give the glucuronic acid derivative. We were able to form protected quaternary ammonium glucosides in three steps. We found that in general only a trifluoromethane sulfonate leaving group would suffice during the quaternisation step. The oxidation gave some desired product, but due to the nature of the aglycone, Amitriptyline, we were unable to selectively oxidise the primary alcohol.

We have now found a synthetic route into quaternary ammonium glucuronides via the condensation reaction between secondary or primary amines and the

hemiacetal. As well as using methyl triflate we have been able to synthesise other primary triflates and use them in the quaternisation reaction successfully.

Deprotection of *O*-acetyl groups was carried out using Na_2CO_3 in MeOH to give mainly the product. When deprotecting the 2,3,4-isobutyryl protected sugar, the 4-*O*-isobutyrate is able to act as a leaving group and undergoes an elimination reaction. Significant 4-*O*-isobutyryl elimination occurs when using Na_2CO_3. When using NaOH or LiOH we see no elimination, but we do see hydrolysis of the aglycone occurring.

We have been able to learn more about the reactivity and stability of N^+-glucuronides during synthesis. The stability of the compounds that have been investigated varies significantly with respect to both purification on silica gel and base deprotection. From my experience of the compounds the order of stability is depicted in figure 4.9.27.

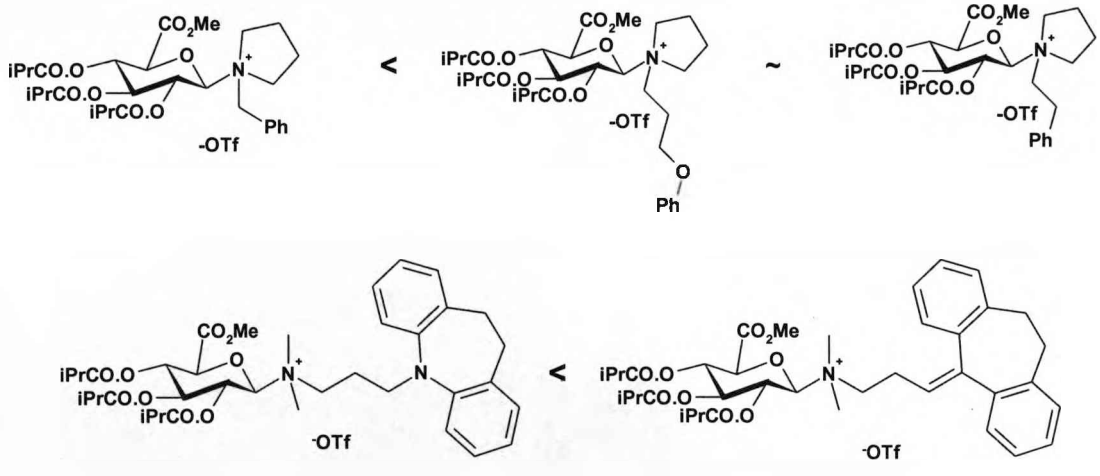
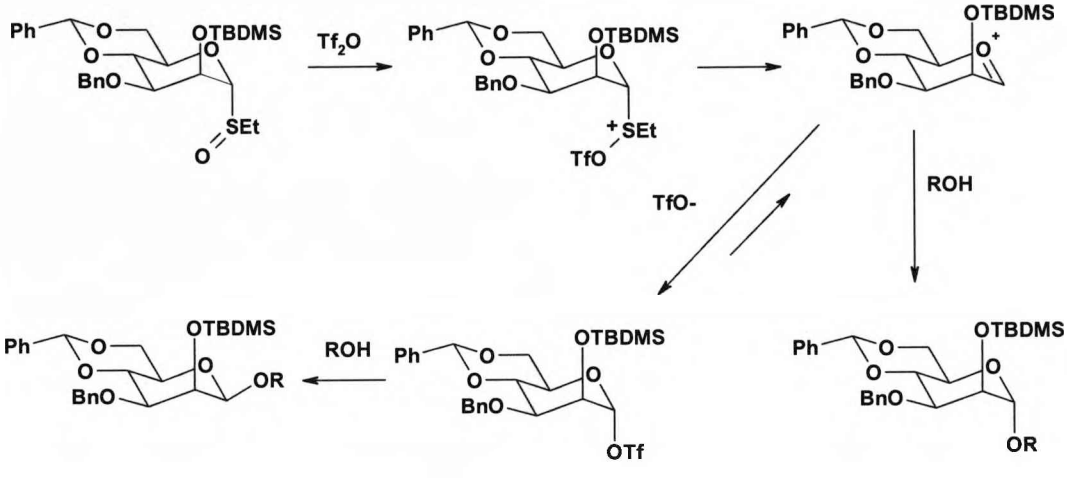


Figure 4.9.27: Quaternary ammonium compounds in order of stability, starting with the least stable

From previous investigations of quaternisation reactions with methyl iodide and methyl triflate, it would appear that for direct quaternisation of a tertiary amine anomeric triflate would be most suitable. Crich *et al.*[66] have used anomeric triflates

to synthesise β-mannosylpyranosides. They designed experiments in order to form either α-linked pyranosides or β-linked pyranosides. To form the β-glycosidic bond they took mannosyl sulfoxide and reacted it with Tf_2O in the presence of 2,6-di-*tert*-butyl-4-methylpyridine, then finally they add the reacting alcohol. This forms the α-triflate, which is then reacted with an alcohol to give a β-configured bond (scheme 5.1.1). To form the more accessible α-manoside the reacting alcohol is added at the start of the reaction.

To form the anomeric triflate, the reactions must be carried out at -78°C in order for stability of the triflate intermediate. They also note the requirement for a non-participating protecting group in the 2- and 3- positions.



Scheme 5.1.1: Reaction conditions used by Crich *et al.*[66] to form anomeric triflate intermediates.

This seems a promising route into quaternary ammonium glucuronides, and should be investigated in a future project.

4.9 References

(1) Bridges, J. W.; Kibby, M. R.; Walker, S. R.; Williams, R. T. *Biochemical Journal* **1968**, *109*, 851.

(2) Zenser, T. V.; Lakshmi, V. M.; Davis, B. B. *Drug Metabolism and Disposition* **1998**, *26*, 856-859.

(3) Pahernik, S. A.; Schmid, J.; Sauter, T.; Schildberg, F. W.; Koebe, H. G. *Xenobiotica* **1995**, *25*, 811-823.

(4) Maguire, J. H.; Butler, T. C.; Dudley, K. H. *Drug Metabolism and Disposition* **1982**, *10*, 595-598.

(5) Borlak, J.; Gasparic, A.; Locher, M.; Schupke, H.; Hermann, R. *Metabolism-Clinical and Experimental* **2006**, *55*, 711-721.

(6) Hiller, A.; Nguyen, N.; Strassburg, C. P.; Li, Q.; Jainta, H.; Pechstein, B.; Ruus, P.; Engel, J.; Tukey, R. H.; Kronbach, T. *Drug Metabolism and Disposition* **1999**, *27*, 605-612.

(7) Hawes, E. M. *Drug Metabolism and Disposition* **1998**, *26*, 830-837.

(8) Zhu, B.; Bush, D.; Doss, G. A.; Vincent, S.; Franklin, R. B.; Xu, S. Y. *Drug Metabolism and Disposition* **2008**, *36*, 331-338.

(9) Chiu, S. H. L.; Huskey, S. E. W. *Drug Metabolism and Disposition* **1998**, *26*, 838-847.

(10) Mey, U.; Wachsmuth, H.; Breyer-Pfaff, U. *Drug Metabolism and Disposition* **1999**, *27*, 1281-1292.

(11) Lebigot, J. F.; Begue, J. M.; Kiechel, J. R.; Guillouzo, A. *Life Sciences* **1987**, *40*, 883-890.

(12) Franklin, R. B. *Drug Metabolism and Disposition* **1998**, *26*, 829-829.

(13) Breyerpfaff, U.; Becher, B.; Nusser, E.; Nill, K.; Baierweber, B.; Zaunbrecher, D.; Wachsmuth, H.; Prox, A. *Xenobiotica* **1990**, *20*, 727-738.

(14) Calligaro, D. O.; Fairhurst, J.; Hotten, T. M.; Moore, N. A.; Tupper, D. E. *Bioorganic & Medicinal Chemistry Letters* **1997**, *7*, 25-30.

(15) Luo, H.; Hawes, E. M.; McKay, G.; Midha, K. K. *Journal of Pharmaceutical Sciences* **1992**, *81*, 1079-1083.

(16) Upadhyaya, P.; McIntee, E. J.; Hecht, S. S. *Chemical Research in Toxicology* **2001**, *14*, 555-561.

(17) Caldwell, W. S.; Greene, J. M.; Byrd, G. D.; Chang, K. M.; Uhrig, M. S.; Debethizy, J. D.; Crooks, P. A.; Bhatti, B. S.; Riggs, R. M. *Chemical Research in Toxicology* **1992**, *5*, 280-285.

(18) Wiener, D.; Doerge, D. R.; Fang, J. L.; Upadhyaya, P.; Lazarus, P. *Drug Metabolism and Disposition* **2004**, *32*, 72-79.

(19) Takitani, S. *Chemical and Pharmaceutical Bulletin* **1959**, *7*, 845.

(20) Hodge, J. E.; Rist, C. E. *Journal of the American Chemical Society* **1952**, *74*, 1498-1500.

(21) Mossine, V. V.; Barnes, C. L.; Mawhinney, T. P. *Journal of Carbohydrate Chemistry* **2007**, *26*, 249-266.

(22) Itoh, A.; Ikuta, Y.; Tanahashi, T.; Nagakura, N. *Journal of Natural Products* **2000**, *63*, 723-725.

(23) Itoh, A.; Ikuta, Y.; Baba, Y.; Tanahashi, T.; Nagakura, N. *Phytochemistry* **1999**, *52*, 1169-1176.

(24) Isbell, H. S.; Frush, H. L. *Journal of Organic Chemistry* **1958**, *23*, 1309-1319.

(25) Simon, H.; Palm, D. *Chemische Berichte-Recueil* **1965**, *98*, 433.

(26) Bognar, R.; Nanasi, P. *Journal of the Chemical Society* **1955**, 185-189.

(27) Baker *J. Chem. Soc.* **1929**, 1206.

(28) Pigman, W.; Nisizawa, K.; Tsuiki, S. *Annual Review of Biochemistry* **1959**, *28*, 15-38.

(29) Zsoldos-Mady, V.; Sohar, P.; Kovacs, J.; Pinter, I.; Szakacs, Z. *Journal of Carbohydrate Chemistry* **2005**, *24*, 19-39.

(30) Jung, M. E.; Yang, E. C.; Vu, B. T.; Kiankarimi, M.; Spyrou, E.; Kaunitz, J. *Journal of Medicinal Chemistry* **1999**, *42*, 3899-3909.

(31) Messaoudi, S.; Anizon, F.; Pfeiffer, B.; Prudhomme, M. *Tetrahedron* **2005**, *61*, 7304-7316.

(32) Messaoudi, S.; Anizon, F.; Pfeiffer, B.; Golsteyn, R.; Prudhomme, M. *Tetrahedron Letters* **2004**, *45*, 4643-4647.

(33) Mutlib, A. E.; Nelson, W. L. *Journal of Pharmacology and Experimental Therapeutics* **1990**, *252*, 593-599.

(34) Kaku, T.; Ogura, K.; Nishiyama, T.; Ohnuma, T.; Muro, K.; Hiratsuka, A. *Biochemical Pharmacology* **2004**, *67*, 2093-2102.

(35) Dalgaard, L. *Acta Chemica Scandinavica Series B-Organic Chemistry and Biochemistry* **1983**, *37*, 923-928.

(36) Skorupowa, E.; Kurszewska, M.; Konitz, A.; Wojnowski, W.; Wisniewski, A. *Carbohydrate Research* **2001**, *331*, 343-346.

(37) P. Karrer, A. S. *Helvetica Chimica Acta* **1921**, *4*, 817-820.

(38) Kaji, E.; Osa, Y.; Takahashi, K.; Hirooka, M.; Zen, S.; Lichtenthaler, F. W. *Bulletin of the Chemical Society of Japan* **1994**, *67*, 1130-1140.

(39) Lergenmuller, M.; Lichtenthaler, F. W. *Carbohydrate Research* **2007**, *342*, 2132-2137.

(40) March J. *March's Advanced Organic Chemistry: Reactions, Mechanisms and Structure*; 6th Edition ed.; Wiley.

(41) Baisch, G.; Ohrlein, R. *Carbohydrate Research* **1998**, *312*, 61-72.

(42) Olofson, R. A.; Schnur, R. C.; Bunes, L.; Pepe, J. P. *Tetrahedron Letters* **1977**, 1567-1570.

(43) Muller, T.; Schneider, R.; Schmidt, R. R. *Tetrahedron Letters* **1994**, *35*, 4763-4766.

(44) Stachulski, A. V. *Tetrahedron Letters* **2001**, *42*, 6611-6613.

(45) Melville, W. **1955**, *10*, 105.

(46) Leroux, J.; Perlin, A. S. *Carbohydrate Research* **1978**, *67*, 163-178.

(47) Araya, I.; Akita, H. *Heterocycles* **2008**, *75*, 1213-1223.

(48) Halcomb, R. L.; Danishefsky, S. J. *Journal of the American Chemical Society* **1989**, *111*, 6661-6666.

(49) Cheshev, P.; Marra, A.; Dondoni, A. *Carbohydrate Research* **2006**, *341*, 2714-2716.

(50) Nicolaou, K. C.; Chucholowski, A.; Dolle, R. E.; Randall, J. L. *Journal of the Chemical Society-Chemical Communications* **1984**, 1155-1156.

(51) Fraser-Reid, B.; Lopez, J. C.; Radhakrishnan, K. V.; Mach, M.; Schlueter, U.; Gomez, A. M.; Uriel, C. *Journal of the American Chemical Society* **2002**, *124*, 3198-3199.

(52) Pitt, N.; Duane, R. M.; O' Brien, A.; Bradley, H.; Wilson, S. J.; O'Boyle, K. M.; Murphy, P. V. *Carbohydrate Research* **2004**, *339*, 1873-1887.

(53) Soli, E. D.; Manoso, A. S.; Patterson, M. C.; DeShong, P.; Favor, D. A.; Hirschmann, R.; Smith, A. B. *Journal of Organic Chemistry* **1999**, *64*, 3171-3177.

(54) Matsubara, K.; Mukaiyama, T. *Chemistry Letters* **1994**, 247-250.

(55) Gyorgydeak, Z.; Thiem, J. *Carbohydrate Research* **1995**, *268*, 85-92.

(56) Shiozaki, M.; Mochizuki, T.; Hanzawa, H.; Haruyama, H. *Carbohydrate Research* **1996**, *288*, 99-108.

(57) Takeda, T.; Sugiura, Y.; Ogihara, Y.; Shibata, S. *Canadian Journal of Chemistry-Revue Canadienne De Chimie* **1980**, *58*, 2600-2603.

(58) Ameijde, J. v. *Perkins transactions 1* **2002**, 1042.

(59) Parekh, H. S.; Marano, R. J.; Rakoczy, E. P.; Blanchfield, J.; Toth, I. *Bioorganic & Medicinal Chemistry* **2006**, *14*, 4775-4780.

(60) Esteves, A. P.; Rodrigues, L. M.; Silva, M. E.; Gupta, S.; Oliveira-Campos, A. M. F.; Machalicky, O.; Mendonca, A. J. *Tetrahedron* **2005**, *61*, 8625-8632.

(61) McCloskey, C. M.; Coleman, G. H. *Journal of Organic Chemistry* **1945**, *10*, 184-193.

(62) Bordwell, F. G.; Brannen, W. T. *Journal of the American Chemical Society* **1964**, *86*, 4645.

(63) Bols, M. *Acta Chemica Scandinavica* **1993**, *47*, 829-834.

(64) Epp, J. B.; Widlanski, T. S. *Journal of Organic Chemistry* **1999**, *64*, 293-295.

(65) Beard, C. D.; Baum, K.; Grakausk.V *Journal of Organic Chemistry* **1973**, *38*, 3673-3677.

(66) Crich, D.; Sun, S. X. *Tetrahedron* **1998**, *54*, 8321-8348.

Chapter Five

Experimental

5.1 Experimental

Moisture sensitive reactions were carried out in anhydrous organic solvents, under a nitrogen atmosphere. For all glucuronide intermediates and products, vacuum rotary evaporation was carried out at <35°C.

^{1}H NMR and ^{13}C NMR were recorded on a Bruker AMX 400 (400 MHz- ^{1}H and 100 MHz- ^{13}C) spectrometer. Chemical shifts (δ) are quoted in ppm and coupling constants (J) are quoted in Hertz (Hz).

Mass spectrometry was carried out on a VG analytical 7070E machine and Fisons TRIO spectrometer, using electron ionisation (EI) and chemical ionisation (CI); a Micromass LCT spectrometer was used for HRMS in the electrospray mode, carried out by Mr. Alan Mills of The University of Liverpool.

Elemental analysis was performed by Mr. Steve Apter of The University of Liverpool using a Thermo Flash EA1112 analyzer, configured for automated elemental analysis.

Infrared spectra were recorded on a Perkin-Elmer 1000 spectrometer or a FTIR-4100 type A instrument in the range of 600-4000cm^{-1}. Samples were prepared as a nujol mull onto NaCl discs and absorption peaks quoted in wavenumbers (cm^{-1}) or prepared as neat solids on the FTIR-4100 instrument.

Melting points were recorded using a Bibby-Sterlin Stuart SMP3 melting point apparatus.

Thin layer chromatography was carried out using Merck 5x2 cm aluminium backed plates with a 0.2mm layer of Kieselgel 60 F_{254}. They were visualised by UV lamp (254nm) and anisaldehyde stain were appropriate. Flash column chromatography was carried out using ICN (230-400 mesh) silica gel. Reverse phase chromatography

was carried out using LiChroprep RP-18 C_{18} silica gel prepared by washing with methanol then water. The solvent used to elute was an increasing gradient of acetonitrile in water.

Preparative HPLC was carried out on a Agilent Technologies 1200 Series Preparative HPLC System, fitted with a Waters XBridge Prep C18 5um (19 x 10mm Guard cartridge) and a Waters XBridge Prep C18 5um OBD (19 x 100mm) column using a neutral gradient of acetonitrile:water.

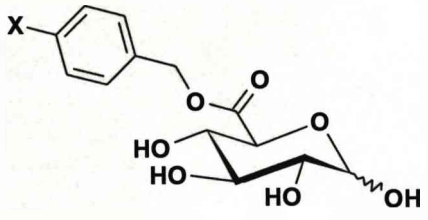
LCMS was carried out by James Maggs of the department of Pharmacology. LCMS was carried out at room temperature on a Beckman Ultrasphere 5-μm C18 column (250 × 4.6 mm i.d.; Beckman Coulter, High Wycombe, Buckinghamshire, U.K.) by gradient elution with acetonitrile (20-45%, v/v, over 20 min) in 0.1% (v/v) formic acid. The eluent flow rate was 0.9 mL/min. Solid materials were dissolved in acetonitrile-water (2:1, v/v) at room temperature, and aliquots of solutions (10 μL) were injected onto the HPLC column without further treatment. The LC system consists of two Jasco PU980 pumps (Jasco UK, Great Dunmow, Essex, UK) and a Jasco HG-980-30 mixing module. Eluted compounds were monitored at 254 nm with a Jasco UV-975 spectrophotometer. Eluate split-flow to the LC-MS interface was *ca.* 40 μL/min. A Quattro II mass spectrometer (Waters Corp, Manchester, UK) fitted with the standard co-axial electrospray source was operated using nitrogen as the nebulizing and drying gas. The interface temperature was 80 °C; electrospray capillary voltage, 3.8 kV; standard cone voltage, 30 V; cone voltage for in-source fragmentation of analytes, 65 V. The instrument was set up for full-scanning acquisitions in the positive-ion mode as follows: *m/z* 50-1050 with a scan time of 5 s and an inter-scan delay of 100 ms. Instrument management and data processing were accomplished through MassLynx 3.5 software.

Optical rotations ($[\alpha]_D$) were carried out on a PerkinElmer polarimeter 343 at a wavelength of 598 nm at 20°C. Solvents and concentrations used are quoted.

5.2 1-β-O-Acyl Glucuronides

-Synthesis of substituted benzyl ester glucuronates

- Using the polymer bound fluoride



X= NO_2 (2.44), MeO (2.49)

To a solution of Glucuronic acid (2g, 10.31 mmol) in DMF (20 ml) at RT under N_2 was added the polymer bound fluoride (5.2g, 15.46 mmol) followed by the substituted benzyl bromide (11.34 mmol). The reaction was left for 24h at RT, after this time the polymer bound fluoride was filtered off and the DMF removed under high vacuum. Column chromatography was carried out eluting with 10-20 % EtOH/DCM.

p-Nitrobenzyl glucuronate (2.44)

Yield: 67 %. Mp: 125-126°C. $[M+Na]^+$ *m/z* 352.0634; $C_{13}H_{15}NO_9Na$ requires *m/z* 352.0645.

^1H NMR δ_H d_6-Acetone (1:1 α/β mixture): 3.19-3.25 (1H, dd, 2β-H, J = 7.7, 9.2 Hz), 3.39-3.48 (2H, dd, 2α-H J = 3.6, 9.1 Hz, t, 3α-H J = 9.1 Hz), 3.65-3.72 (3H, t, 4α-H J = 9.1 Hz, t, 4β-H, t, 3β-H), 3.96-4.00 (1H, d, 5α-H, J = 9.8 Hz), 4.40-4.43 (1H, d, 5β-H J = 9.4 Hz), 4.60-4.64 (1H, d, 1β-H, J = 7.8 Hz), 5.19-5.22 (1H, d, 1α-H J = 3.6 Hz), 5.38-5.40 (4H, 2 x s, CO_2CH_2), 7.71-7.74 (4H, dd, α/β-ArH), 8.24-8.27 (4H, dd, α/β-ArH).

^{13}C NMR δ_C d_6-Acetone: 66.0, 66.1, 70.2, 73.2, 73.3, 73.5, 73.7, 74.6, 76.3, 77.1, 77.7, 78.8, 79.9, 84.8, 94.5, 99.2, 124.5, 124.7, 128.2, 129.6, 145.2, 170.7. *m/z* (ESI, +ve ion mode) 352 $[MNa]^+$, 100 %.

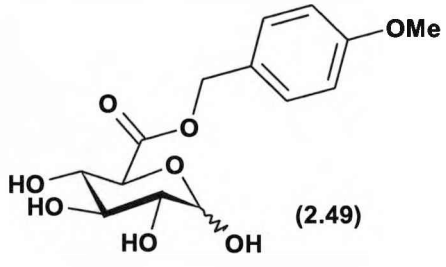
p-Methoxybenzyl glucuronate (2.49)

Yield: 58 %. Found: C, 51.9; H, 5.6 %; $[M+Na]^+$ *m/z*, 337.0905. $C_{14}H_{18}O_8$. $0.5.H_2O$ requires C, 52.0 ; H, 5.9 %; $C_{14}H_{18}O_8Na$ requires *m/z*, 337.0899.

^1H NMR δ_H d_6-Acetone (1:1 α/β mixture): 3.22 (1 H, m, 2β-H), 3.35-3.45 (2 H, m, 2α-H, 3β-H), 3.65 (2 H, H-4 α and β, J = 9.0 Hz), 3.73 (1 H, t, 3α-H, J = 8.9 Hz), 3.86 (1 H, d, H-5β, J = 9.8 Hz), 4.31 (1 H, 5α-H, J = 9.3 Hz), 4.57 (1 H, d, H-1β, J = 7.7 Hz), 5.10-5.20 (4 H, $ArCH_2O$ α and β), 5.17 (1 H, d, H-1α, J = 3.1 Hz), 6.95 and 7.36 (8 H, approx. dd, ArH).

^{13}C NMR δ_C d_6-Acetone: 56.0, 56.3, 67.2, 67.3, 73.2, 73.3, 73.6, 74.5, 76.2, 77.2, 94.3, 99.0, 115.1, 115.7, 129.3, 129.4, 131.1, 131.2, 136.1, 161.1, 162.7, 170.2 and 171.0. *m/z* (ES, +ve ion mode) 337 $[MNa]^+$, 100 %.

-Using tetra-n-butylammonium fluoride (TBAF)



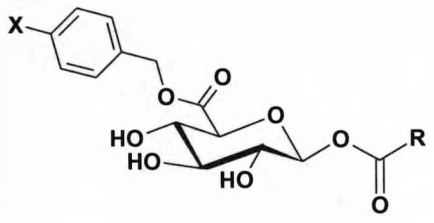
(2.49)

A solution of Glucuronic acid (2g, 10.31 mmol) in DMF (20 ml) was stirred at RT under N_2, TBAF (10.84 ml, 10.31 mmol) was added and the reaction left stirring for 1h. To this was added *p*-methoxybenzyl bromide (1.56 ml, 11.34 mmol) to the reaction and the solution left for a further 16h. The DMF was then removed in *vacuo*. Column chromatography was carried out using 10-20 % IPA/DCM. The foam was re-crystallised from warmed DCM, with the addition of ether on cooling.

p-Methoxybenzyl glucuronate (2.49)

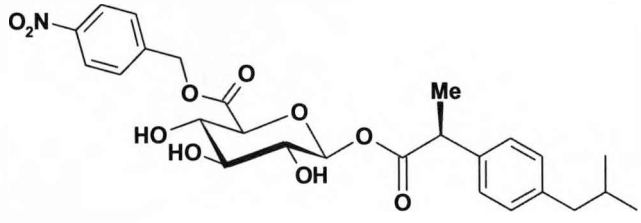
Yield: 72 %. data agrees with previous synthesis

6.2.2 -General coupling method using HATU



To a solution of Glucuronate ester **(2.44** or **2.49)** (0.32 mmol) in acetonitrile (4 ml) was added, HATU (133 mg, 0.35 mmol), carboxylic acid (0.35 mmol) and NMM (0.07 ml, 0.64 mmol), the reaction was then left stirred at RT under N_2 for 3-4 hrs monitored by TLC. The reaction was then quenched with Amberlite IR-120 H^+ ion-exchange resin (337 mg, 0.64 mmol), until pH 6-7 was reached. The resin was then filtered off and the acetonitrile removed in *vacuo*. Column chromatography was used to purify, eluting with 5-10 % IPA/DCM.

p-Nitrobenzyl 1-(α-methyl-4-isobutylphenyl)acetyl-β-D-glucopyranuronate (2.57)



Yield: 32%

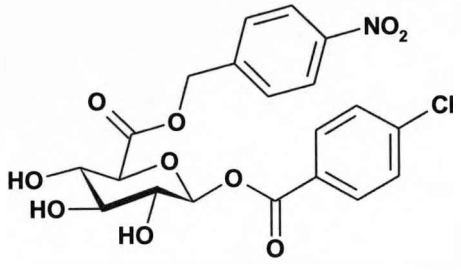
Mp: 141-142°C. Found: C, 60.43, H, 6.09, N, 2.66 %; $[M+Na]^+$ *m/z*, 540.1862. $C_{26}H_{31}NO_{10}$ requires C, 60.34 H, 6.03, N, 2.71 %; $C_{26}H_{31}NO_{10}Na$ requires *m/z* 540.1846

^1H NMR δ_H d_6 Acetone: 0.83-0.89 (6H, d, $CH(C\underline{H}_3)_2$), 1.43-1.47 (3H, d, $CHC\underline{H}_3$ J = 7.2 Hz), 1.75-1.89 (1H, m, $C\underline{H}(CH_3)_2$), 2.4-2.6 (2H, d, $C\underline{H}_2CH$ J = 7.2 Hz), 3.28-3.35 (1H, t, 2-H, J = 8.7, 8.3 Hz), 3.83-3.50 (1H, t, 3-H, J = 8.9 Hz), 3.51-3.59 (1H, t, 4-H, J = 9.1 Hz), 3.65-3.75 (1H, q, $C\underline{H}CH_3$, J = 7.1 Hz), 3.98-4.10 (1H, d, 5-H J = 9.2 Hz), 5.20-5.25

(2H, AB qt, $CO_2C\underline{H}_2$ J = 13.9 Hz), 5.48-5.53 (1H, d, 1-H J = 8.1 Hz), 7.05-7.16 (2H, d, ArH), 7.20-7.31 (2H, d, ArH), 7.59-7.66 (2H, d, ArH), 8.16-8.22 (2H, d, ArH)

^{13}C NMR δ_C d_6-Acetone: 19.5, 19.8, 23.0, 45.8, 45.9, 66.2, 73.0, 73.9, 77.4, 77.6, 96.0, 124.9, 128.4, 129.5, 130.3, 138.9, 141.6, 144.9, 148.9, 169.2, 174.1; *m/z* (ES, +ve ion mode) 540 ($M+Na^+$), 100 %.

p-Nitrobenzyl 1-(4-chlorobenzoyl)-β-D-glucopyranuronate (2.46)



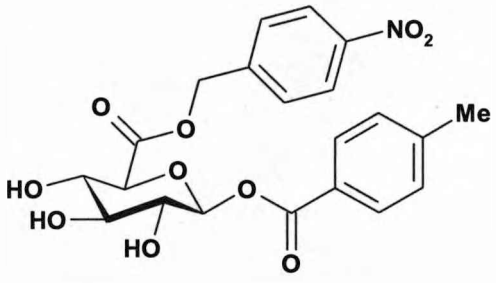
Yield: 44%

Found $[M+Na]^+$ *m/z* 490.0517 $C_{20}H_{18}NO_{10}{}^{35}ClNa$ requires *m/z* 490.0497

^1H NMR δ_H d_6-Acetone: 3.50-3.62 (2H, m, 2-H, 3-H), 3.63-3.71 (1H, m, 4-H), 4.10-4.13 (1H, d, 5-H, J = 9.6 Hz), 4.53 (1H, broad s, O$\underline{H}$), 4.62 (1H, broad s, O$\underline{H}$), 4.76 (1H, broad s, O$\underline{H}$), 5.21-5.25 (2H, d, $CO_2C\underline{H}_2$), 5.70-5.74 (1H, d, 1-H, J=7.6 Hz), 7.40-7.44 (2H, d, Ar$\underline{H}$), 7.53-7.58 (2H, d, Ar$\underline{H}$), 7.91-7.96 (2H, d, Ar$\underline{H}$), 8.04-8.09 (2H, d, Ar$\underline{H}$).

^{13}C NMR δ_C d_6-Acetone: 66.3, 73.1, 73.9, 77.3, 77.5, 96.5, 129.4, 129.7, 130.1, 130.3, 132.7, 132.8, 144.8, 149.0, 165.1, 169.3. *m/z* (ES, +ve ion mode) 490 $[M+Na]^+$ 100 %

p-Nitrobenzyl 1-(4-methylbenzoyl)-β-D-glucopyranuronate (2.59)

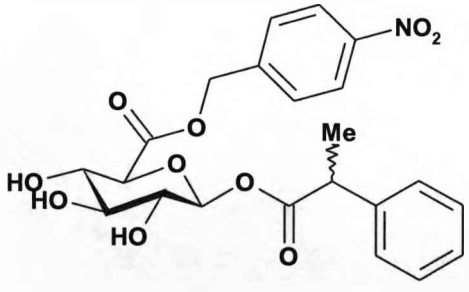


Yield: 52 %. Found: $[M+Na]^+$ *m/z* 470.1053; C, 56.2, H, 4.78, N, 3.09 %; $C_{21}H_{21}O_{10}NNa$ requires *m/z* 470.1063; $C_{21}H_{21}O_{10}N$ requires C, 56.4, H, 4.73, N, 3.13 %.

^1H NMR δ_H d_6-Acetone: 2.42 (3H, s, $ArCH_3$), 3.64-3.73 (2H, t, 2-H, J = 7.9 Hz, t, 3-H, J = 8.9 Hz), 3.77-3.81 (1H, t, 4-H, J = 9.1 Hz), 4.24-4.26 (1H, d, 5-H, J = 9.6 Hz), 5.37-5.39 (2H, AB qt, CH_2Ar), 5.84-5.86 (1H, d, 1-H, J = 7.8 Hz), 7.32-7.37 (2H, d, ArH), 7.69-7.72 (2H, d, ArH), 7.96-8.01 (2H, d, ArH), 8.20-8.23 (2H, d, ArH).

^{13}C NMR δ_C d_6-Acetone: 20.8, 65.2, 71.9, 72.8, 76.3, 76.3, 95.0, 113.8, 123.5, 126.8, 128.4, 129.7, 130.0, 143.7, 144.6, 164.7, 168.1. *m/z* (ES +ve ion mode) 470 100 %.

p-Nitrobenzyl 1-(α-methylphenyl)acetyl-β-D-glucopyranuronate (2.60)



Yield: 50 %, Reaction started with *(S)*-2-phenylpropanoic acid, racemisation occurs *in situ;* it is presumed that the major component is the *(S)*-isomer.

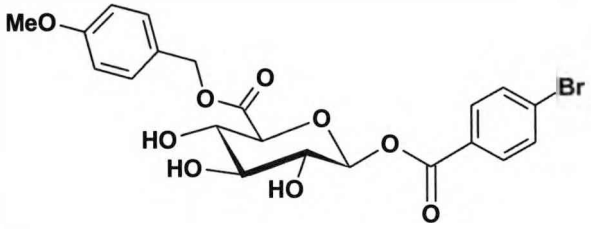
Diastereoisomers not separated.

Found $[M+Na]^+$ *m/z* 484.120; $C_{22}H_{23}NO_{10}Na$ requires *m/z* 484.122.

^{1}H NMR δ_H d_6-Acetone ratio *R/S*-isomers 1:4: 1.43-1.45 (3H, d, *R*-isomer CH($C\underline{H}_3$), J = 7.1 Hz), 1.46-1.48 (3H, d, *S*-isomer CH($C\underline{H}_3$), J = 7.1 Hz), 3.42-3.46 (1H, t, *R* and *S*-isomer 2-H, J = 8.7, 8.2 Hz), 3.58-3.62 (1H, t, *R* and *S*-isomer 3-H, J = 9.0 Hz), 3.64-3.69 (1H, t, *R* and *S*-isomer 4-H, J = 9.0 Hz), 3.70-3.75 (1H, q, *R*-isomer $C\underline{H}(CH_3)$), 3.83-3.88 (1H, q, *S*-isomer $C\underline{H}(CH_3)$, J = 7.1 Hz), 4.11-4.14 (1H, d, *R* and *S*-isomer 5-H, J = 9.4 Hz), 5.30-4.40 (2H, AB qt, *R* and *S*-isomer $C\underline{H}_2Ar$), 5.62-5.64 (1H, d, *R* and *S*-isomer 1-H, J = 8.2 Hz), 7.27-7.36 (5H, m, ArH), 7.65-7.67 (2H, d, ArH), 8.20-8.22 (2H, d, ArH).

^{13}C NMR δ_C d_6-Acetone: 19.5, 19.7, 46.2, 46.3, 66.2, 73.0, 73.8, 77.4, 77.4, 95.9, 124.7, 128.3, 129.8, 129.5, 129.8, 141.6, 144.9, 148.9, 169.2, 173.9. *m/z* (ES +ve ion mode) 484 100 %.

p-Methoxybenzyl 1-(4-bromobenzoyl)-β-D-glucopyranuronate (2.61)



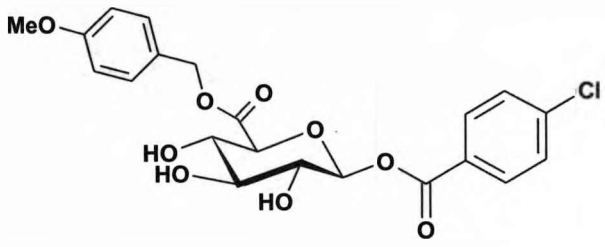
Yield: 45 %

Found: C, 50.55, H, 4.29 %; $[M+Na]^+$ *m/z* 519.0259. $C_{21}H_{21}O_9{}^{79}Br$ requires C, 50.72, H, 4.25 %; $C_{21}H_{21}O_9{}^{79}BrNa$ requires *m/z* 519.0267.

^{1}H NMR δ_H d_6-Acetone: 3.40-3.54 (2H, m, 2-H, 3-H), 3.56-3.62 (1H, m, 4-H), 3.61-3.66 (3H, s, $OC\underline{H}_3$), 3.97-4.01 (1H, d, 5-H, J = 9.5 Hz), 4.96-5.01 (2H, AB qt, $CO_2C\underline{H}_2$), 5.65-5.69 (1H, d, 1-H, J=7.8 Hz), 6.73-6.78 (2H, m, Ar$\underline{H}$), 7.16-7.22 (2H, m, Ar$\underline{H}$), 7.56-7.61 (2H, m, Ar$\underline{H}$), 7.83-7.88 (2H, m, Ar$\underline{H}$).

^{13}C NMR δ_C d_6-Acetone: 55.1, 66.7, 72.2, 73.1, 76.5, 76.8, 95.6, 114.2, 128.2, 128.5, 129.0, 130.4, 131.9, 132.3, 145.2, 164.3, 168.5. *m/z* (ES, +ve ion mode) 519 $[M+Na]^+$ 100 %.

p-Methoxybenzyl 1-(4-chlorobenzoyl)-β-D-glucopyranuronate (2.62)



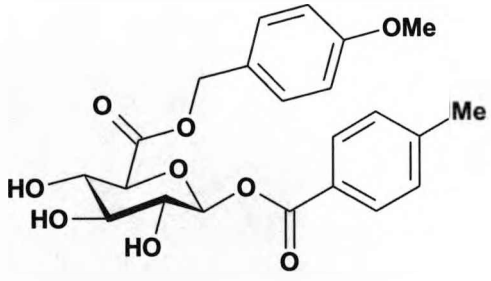
Yield: 55 %

Mp: 182-183°C. Found: $[M+Na]^+$ *m/z* 475.0755 $C_{21}H_{21}O_9{}^{35}ClNa$ requires *m/z* 475.0772

^1H NMR δ_H d_6-Acetone: 3.62-3.69 (2H, m, 2-H, 3-H), 3.73-3.78 (1H, m, 4-H), 3.79 (3H, s, OCH_3), 4.13-4.17 (1-H, d, 5-H, J = 9.5 Hz), 5.14 (2H, s, OCH_2), 5.82-5.84 (1H, d, 1-H, J = 7.9 Hz), 6.89-6.93 (2H, m, ArH), 7.33-7.37 (2H, m, ArH), 7.58-7.61 (2H, m, ArH), 8.08-8.11 (2H, m, ArH)

^{13}C NMR δ_C d_6-Acetone: 55.9, 67.6, 73.0, 73.9, 77.3, 77.6, 96.1, 115.0, 115.7, 128.0, 129.1, 130.5, 131.1, 131.3, 135.9, 165.8, 169.5. *m/z* (ES +ve ion mode) 475 $[M+Na]^+$ 100 %.

p-Methoxybenzyl 1-(4-methylbenzoyl)-β-D-glucopyranuronate (2.63)



Yield: 45 %

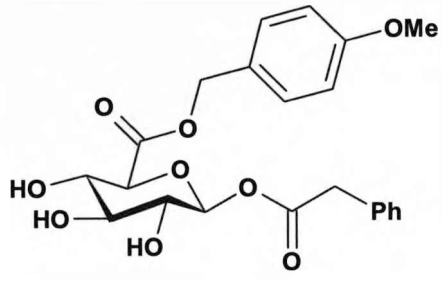
Found: C, 58.44 %, H, 5.54 %; [M+Na] *m/z* 455.133. $C_{22}H_{24}O_9.H_2O$ requires C, 58.66 %, H, 5.81 %, $C_{22}H_{24}O_9Na$ requires *m/z* 455.132.

^1H NMR δ_H d_6-Acetone: 2.39 (3H, s, CCH_3), 3.47-3.52 (2H, m, 2-H, 3-H), 3.55-3.64 (1H, m, 4-H), 3.74 (3H, s OCH_3), 3.99-4.01 (1H, d, 5-H, J = 9.4 Hz), 4.97-5.00 (2H, AB

qt, OCH_2), 5.65-5.68 (1H, d, 1-H, J = 8.0 Hz), 6.74-6.78 (2H, d, ArH), 7.17-7.22 (4H, d, ArH), 7.81-7.84 (2H, d, ArH).

^{13}C NMR δ_C d_6-DMSO: 21.5, 55.4, 66.5, 71.6, 72.4, 75.6, 76.3, 95.0, 114.2, 126.4, 127.8, 129.7, 130.0, 130.3, 144.7, 159.6, 164.8, 168.7. *m/z* (ES +ve ion mode) 455 $[M+Na]^+$ 100 %.

p-Methoxybenzyl 1-(2-phenyl)acetyl-β-D-glucopyranuronate (2.64)



Yield: 55 %.

Found: C, 60.35, H, 5.7 %; $[M+Na]^+$ *m/z*, 455.1321. $C_{22}H_{24}O_9.0.5H_2O$ requires C, 59.9, H, 5.7 %; $C_{22}H_{24}O_9Na$ requires *m/z*, 455.1318.

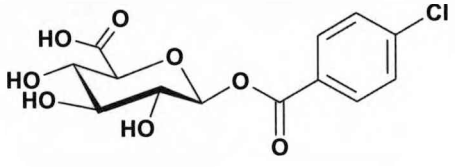
^1H NMR δ_H d_6-Acetone: 3.32 (1 H, t, 2-H, J = 8.5 Hz), 3.43 (1 H, t, 3-H, J = 9.0 Hz), 3.54 (1 H, t, 4-H, J = 9.3 Hz), 3.60 (2 H, s, $PhCH_2CO$), 3.66 (3 H, s, CH_3O), 3.88 (1 H, d, 5-H, J = 9.7 Hz), 5.10-5.18 (2 H, AB qt, $ArCH_2O$), 5.58-5.60 (1H, d, 1-H, J = 8.1 Hz), 6.77 and 7.20 (4 H, dd, ArH of PMB ester) and 7.10-7.20 (5 H, m, ArH of $PhCH_2$).

^{13}C NMR δ_C d_6-Acetone: 41.5, 56.0, 67.6, 73.1, 73.9, 77.6 (x2), 96.0, 115.1, 128.2, 129.2, 129.6, 130.7, 131.2, 135.2, 161.1, 169.4, 171.1. *m/z* (ES +ve mode) 455 $[MNa]^+$, 100%.

6.2.3 -Deprotection using hydrogenation of the PNB group under acidic conditions.

To a solution of *p*-nitrobenzyl protected AG (**(2.46)** or **(2.47)**) (0.3 mmol) in iPrOH/THF (3 ml) (2:1) was added, Pd/C (64 mg, 20 mol %) and Amberlite IR-120 H^+ ion exchange resin (631 mg, 1.2 mmol). The reaction was then left stirring for 30 mins at RT under H_2(g). The Pd/C was then filtered off onto a pad of cellite and the solvent removed *in vacuo.* The residue was then triturated with DCM.

***1-(4-chlorobenzoyl)-β-D-glucopyranuronic acid*[1]**



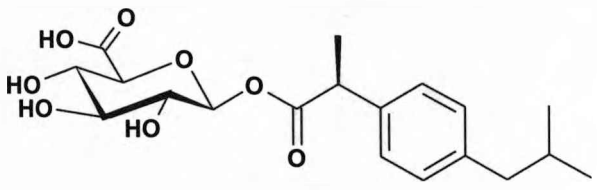
Yield: 94 %

$[\alpha]^{25}_D$ = -25.00 (c = 0.008 gml^{-1} in acetone). Found $[M-H]^-$ *m/z* 331.0221 $C_{13}H_{12}O_8{}^{35}Cl$ requires *m/z* 331.0226

^1H NMR δ_H d_6-Acetone: 3.61-3.76 (3H, m, 2-H, 3-H, 4-H), 4.07-4.12 (1H, d, 5-H, J = 9.5 Hz), 5.81-5.84 (1H, d, 1-H, J = 7.9 Hz), 7.72-7.76 (2H, m, ArH), 7.99-8.04 (2H, m, ArH).

^{13}C NMR δ_C d_6-Acetone: 72.9, 73.8, 77.1, 77.4, 96.4, 129.4, 129.9, 132.8, 133.2, 165.1, 170.2. *m/z* (ES –ve ion mode) 331 $[M-H]^-$ 100 %.

1-((S)-α-methyl-4-isobutylphenyl)acetyl-β-D-glucopyranuronic acid[1](2.54)



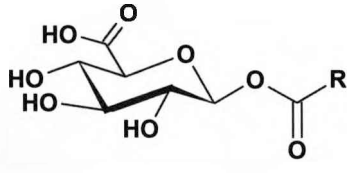
Yield: 78 %

Found $[M-H]^-$ *m/z* 381.1559 $C_{19}H_{25}O_8$ requires *m/z* 381.1549

^1H NMR δ_H d_6-Acetone: 0.85-0.91 (6H, d, $CH(C\underline{H}_3)_2$), 1.43-1.48 (3H, d, $CHC\underline{H}_3$, J= 7.0 Hz), 1.77-1.90 (1H, m, $C\underline{H}(CH_3)_2$), 2.43-2.47 (2H, d, $C\underline{H}_2CH$, J =7.0 Hz), 3.41-3.48 (1H, t, 2-H, J = 8.3 Hz), 3.55-3.68 (2H, t, 3-H, J = 8.7 Hz, t, 4-H, J = 9.1 Hz), 3.78-3.85 (1H, q, $C\underline{H}CH_3$, J = 7.2 Hz), 3.94-3.99 (1H, d, 5-H J = 9.3 Hz), 5.58-5.61 (1H, d, 1-H, J = 8.0 Hz), 7.08-7.13 (2H, d, ArH), 7.21-7.26 (2H, d, ArH).

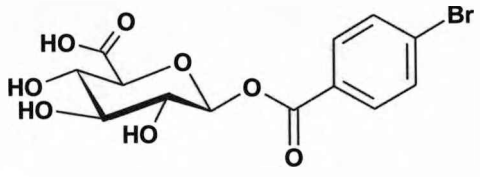
^{13}C NMR δ_C d_6-Acetone: 18.6, 21.9, 30.1, 38.2, 45.5, 71.8, 72.6, 75.9, 76.4, 94.7, 125.2, 127.4, 129.3, 137.8, 140.4, 172.9. *m/z* (ES –ve ion mode) 381 $[M-H]^-$ 100 %.

Deprotection of the PMB group using acid hydrolysis.



To the PMB ester (91 mg, 0.21 mmol) was added a solution of 10 % TFA (90 % in water) in DCM (0.91 ml) at 0°C, and the reaction left for 30mins to 3h depending on the AG (monitored by TLC). The reaction solvent was then removed *in vacuo*, and the residue triturated with DCM.

1-(4-bromobenzoyl)-β-D-glucopyranuronic acid[1] (2.57)



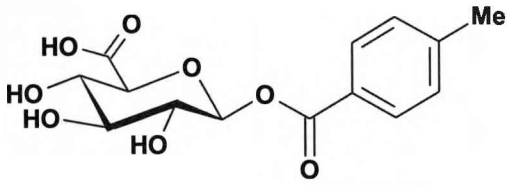
Yield: 52%

Found: C 41.70, H 3.63 %; $[M-H]^-$ *m/z* 374.9723. $C_{13}H_{13}O_8Br$ requires C 41.40, H 3.47 %; $C_{13}H_{12}O_8{}^{79}Br$ requires *m/z* 374.9716

^1H NMR δ_H d_6-Acetone: 3.60-3.76 (3H, m, 2-H, 3-H, 4-H), 4.08-4.13 (1H, d, 5-H J = 9.3 Hz), 5.81-5.84 (1H, d, 1-H, J = 7.6 Hz), 7.72-7.77 (2H, d, ArH), 7.99-8.04 (2H, d, ArH).

^{13}C NMR δ_C d_6-Acetone: 72.9, 73.8, 77.1, 77.4, 96.4, 129.4, 129.9, 132.8, 133.1, 160.5, 170.2. *m/z* (ES, -ve ion mode) 375, $[M-H]^-$ 100 %.

1-(4-methylbenzoyl)-β-D-glucopyranuronic acid[1] (2.53)



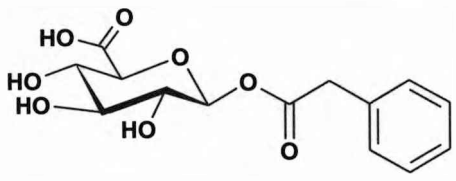
Yield: 90%

Mp: 168-169°C. Found: [M-H] *m/z* 311.0769; C, 53.63, H, 5.29 %. $C_{14}H_{15}O_8$ requires *m/z* 311.0767; $C_{14}H_{16}O_8$ requires C, 53.85, H, 5.16 %.

^1H NMR δ_H d_6-Acetone: 2.42 (3H, s, $ArCH_3$), 3.60-3.80 (3H, m, 2-H, 3-H, 4-H), 4.08-3.97-4.11 (1H, d, 5-H, J = 9.5 Hz), 5.81-5.83 (1H, d, 1-H, J = 8.0 Hz), 7.18-7.22 (2H, d, ArH), 7.81-7.87 (2H, d, ArH).

^{13}C NMR δ_C d_6-Acetone: 22.0, 73.0, 73.9, 77.1, 77.6, 96.1, 126.4, 128.1, 130.9, 131.1, 165.8, 170.4. *m/z* (ES, -ve ion mode) 311 $[M-H]^-$ 100 %.

1-(2-phenyl)acetyl-β-D-glucopyranuronate[2](2.55)



Yield: 82%

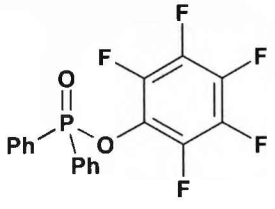
Found $[M-H]^-$ *m/z* 311.0767 $C_{14}H_{15}O_8$ requires *m/z* 311.0761.

^1H NMR δ_H d_6-Acetone: 3.32-3.38 (1H, t, 2-H J = 9.1 Hz), 3.43-3.50 (1H, t, 3-H, J = 9.3 Hz), 3.52-3.59 (1H, t, 4-H J = 9.5 Hz) 3.61 (2H, s, CH_2), 3.83-3.88 (1H, d, 5-H, J = 9.5 Hz), 5.45-5.51 (1H, d, 1-H, J = 8.0 Hz), 7.15-7.20 (5H, m, ArH).

^{13}C NMR δ_C d_6-Acetone: 41.5, 72.9, 73.8, 77.1, 77.6, 95.9, 128.2, 129.5, 130.8, 135.2, 170.4, 171.2. *m/z* (ES –ve ion mode) 311 $[M-H]^-$ 100 %.

6.2.4 -Other Coupling reagents

-Pentafluorophenyl diphenylphosphinate (2.62)

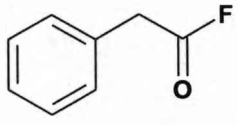


Diphenylphosphinic chloride (2.99 ml, 15.7 mmol) was added to a solution of pentafluorophenol (2.89 g, 15.7 mmol) in DCM (30ml). A solution of imidazole (1.07 g, 15.7 mmol) in DCM was then added dropwise to the reaction at RT, the reaction was then left for 1h. The imidazole salt was then filtered off and the filtrate concentrated *in vacuo*. A column was run using 20-40 % EtOAc/Hexane. The product was then stored in a desiccator for 2 weeks to give a white solid[3].

^{31}P {H} NMR d_6-Acetone: 37.528ppm

The synthesis of acid fluorides

2-Phenylacetyl fluoride (2.77)[4]

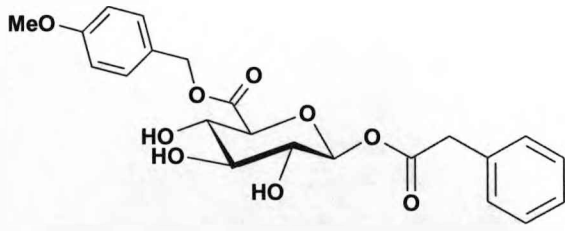


A solution of DCM (2ml) and phenyl acetic acid (48 mg, 0.35 mmol) was cooled to -20^{0}C. Pyridine (0.032 ml, 0.39 mmol) was added to the stirring solution followed by cyanuric fluoride (0.1 ml, 0.78 mmol)[5]. The reaction was allowed to reach -10^{0}C, and left stirring for 2h. The reaction was diluted with DCM (10 ml); this was then extracted with water (3 x 10 ml). The DCM layer was then dried over $MgSO_4$ and concentrated to ~2ml.

IR ν_{max} (cm^{-1}) (nujol mull): 1844.8 (s, C=O stretch), 1456 (s, aromatic C=C stretch), 1077.9 (s, C-O stretch).

Coupling method using acid fluorides.

p-Methoxybenzyl 1-(2-phenyl)acetyl-β-D-glucopyranuronate (2.64)



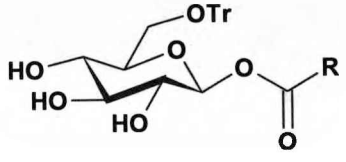
A solution of PMB glucuronate (100 mg, 0.32 mmol) in acetonitrile (2 ml) was cooled to -10^{0}C and evacuated with N_2. To this was added the NMM (0.035 ml, 0.32 mmol), followed by the acid fluoride (0.93 ml, (0.35M in DCM) 0.32 mmol). The

reaction was left for 6.5h at -10°C and then the solvent was removed *in vacuo*. Column chromatography was carried out using 5-10 % EtOH/DCM.

Yield 31 %, data as before with previous HATU coupling method (p. 172).

5.3 The synthesis of Acyl glucosides

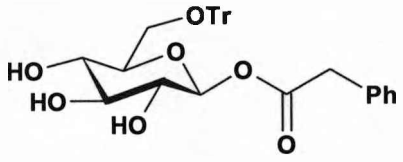
-Coupling method using HATU



To a solution of 6-*O*-trityl glucose[6] (300 mg, 0.71 mmol) in MeCN (8 ml) was added HATU (298 mg, 0.78 mmol), and the respective carboxylic acid (0.78 mmol). The reaction was then flushed with N_2 and the NMM (0.16 ml, 1.42 mmol) added whilst stirring at RT. After 4hrs of stirring at RT the reaction was quenched with the addition of Amberlite H^+resin (747 mg, 1.42 mmol) for 30 mins. The resin was then filtered off and washed with MeCN. The solvent was then removed *in vacuo* and the residue purified by column chromatography using 5 % EtOH/DCM.

N.B. For the 2-phenyl propionoic acid derivative we used the racemic acid, and separated the diastereoisomers by chromatography. The α-dimethyl substituted carboxylic acid required a longer reaction time (16 hrs).

6-O-Trityl 1-(2-phenyl)-acetyl-β-D-glucopyranose (3.14)



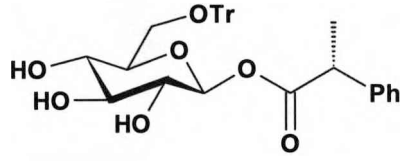
Yield: 55 %

Found: $[M+Na]^+$ *m/z* 563.205 $C_{33}H_{32}O_7Na$ requires *m/z* 563.204.

^1H NMR δ_H CD_3CN: 3.12-3.16 (1H, dd, 6-H, J = 10.2, 5.7 Hz), 3.22-3.25 (1H, dd, 6-H', J = 10.2, 2.0 Hz), 3.34-3.38 (2H, m, 2-H, 4-H), 3.53-3.55 (2H, m, 3-H, 5-H), 3.77 (2H, s, $C\underline{H}_2Ph$), 5.49-5.51 (1H, d, 1-H, J = 7.8 Hz), 7.22-7.34 (14H, m, ArH), 7.41-7.44 (6H, m, ArH).

^{13}C NMR δ_C CD_3CN: 17.4, 40.1, 56.6, 62.8, 69.5, 72.2, 75.8, 76.2, 85.8, 95.3, 126.7, 127.4, 128.2, 129.1, 133.6, 143.7, 170.1. *m/z* (ES +ve ion mode) $[M+Na]^+$ 100 %.

6-O-Trityl 1-([(2R)-phenyl]propanoyl)-β-D-glucopyranose (3.15)



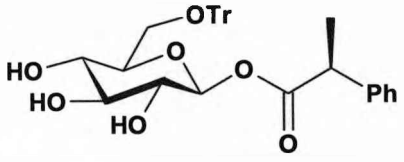
Yield: 53 %

Found: C 70.6 %, H 6.58 %; [M+Na] *m/z* 577.220. $C_{34}H_{34}O_7.H_2O$ requires C 71.31 %, H 6.33 %; $C_{34}H_{34}O_7Na$ *m/z* 577.220.

^1H NMR δ_H d_6-Acetone: 1.54-1.56 (3H, d, $CH(C\underline{H}_3)$, J = 7.2 Hz), 3.24-3.28 (1H, dd, 6-H', J = 9.9, 4.2 Hz), 3.38-3.41 (1H, dd, 6-H, J = 9.9, 2.0 Hz), 3.42-3.50 (2H, m, 2-H, 4-H), 3.57-3.64 (2H, m, 3-H, 5-H), 3.87-3.93 (1H, q, $C\underline{H}(CH_3)$), 4.23 (1H, m, O$\underline{H}$), 4.46 (1H, d, O$\underline{H}$), 4.49 (1H, m, O$\underline{H}$), 5.58-5.59 (1H, d, 1-H, J = 7.8 Hz), 7.21-7.39 (12H, m, ArH), 7.40-7.41 (2H, m, ArH), 7.50-7.52 (6H, m, ArH).

^{13}C NMR δ_C d_6-Acetone: 19.9, 46.5, 64.7, 71.7, 74.2, 77.7, 78.7, 87.4, 96.2, 128.2, 128.2, 128.9, 128.9, 129.8, 130.1, 141.8, 145.6, 174.2. *m/z* (ES +ve ion mode) $[M+Na]^+$ 100 %

6-O-Trityl 1-([(2S)-phenyl]propanoyl)-β-D-glucopyranose (3.16)

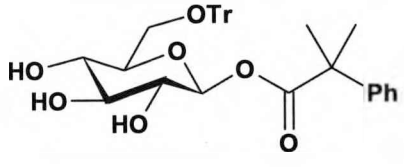


Yield: 53 %

^{1}H NMR δ_H d_6-Acetone: 1.49-1.50 (3H, d, CH(C<u>H</u>$_3$) J = 7.1 Hz), 3.16-3.20 (1H, dd, 6-H', J = 9.9, 5.9 Hz), 3.34-3.37 (1H, dd, 6-H, J = 9.9, 2.0 Hz), 3.38-3.50 (2H, m, 2-H, 4-H), 3.55-3.62 (2H, m, 3-H, 5-H), 3.88-3.94 (1H, q, C<u>H</u>(CH$_3$)), 5.60-5.62 (1H, d, 1-H, J = 7.8 Hz), 7.20-7.38 (12H, m, ArH), 7.40-7.41 (2H, m, ArH), 7.50-7.52 (6H, m, ArH).

^{13}C NMR δ_C d_6-Acetone: 20.1, 46.4, 64.6, 71.6, 74.2, 77.7, 78.6, 87.3, 96.2, 128.1, 128.2, 128.8, 128.9, 129.9, 130.0, 142.0, 145.6, 174.1.

6-O-Trityl 1-(2,2-dimethylphenyl)acetyl-β-D-glucopyranose (3.17)



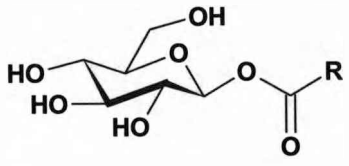
Yield: 49 %

Found: $[M+Na]^+$ *m/z* 591.283. $C_{35}H_{36}O_7Na$ requires *m/z* 591.236.

^{1}H NMR δ_H CD_3CN: 1.58 (3H, s, C(C<u>H</u>$_3$)), 1.64 (3H, s, C(C<u>H</u>$_3$)), 3.0-3.14 (1H, dd, 6-H), 3.21-3.38 (3H, m, 6-H', 2-H, 4-H), 3.51-3.56 (2H, m, 3-H, 5-H), 5.51-5.53 (1H, d, 1-H, J = 8.1 Hz), 7.22-7.32 (14H, m ArH), 7.40-7.45 (6H, m, ArH).

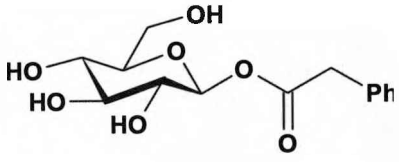
^{13}C NMR δ_C CD_3CN: 25.1, 26.3, 46.3, 62.7, 69.7, 72.1, 75.7, 76.3, 85.8, 94.3, 125.4, 126.5, 126.7, 127.5, 128.1, 128.3, 143.7, 144.1, 174.9. *m/z* (ES +ve ion mode) $[M+Na]^+$ 100 %.

Deprotection of the 6-O-trityl group by acid hydrolysis



To a solution of the trityl protected compound (0.4 mmol) in DCM (4 ml) was added water (0.014 ml, 0.8 mmol) followed by TFA (0.029 ml, 0.4 mmol) with vigorous stirring at RT. The reactions were monitored by TLC and showed completion in ~30 mins. The solvent was removed *in vacuo* and the residue purified by column chromatography using 10-20 % EtOH/DCM.

1-(2-phenyl)acetyl-β-D-glucopyranose (3.18)



Yield: 92 %

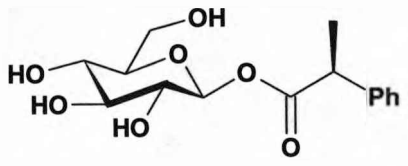
Found: C, 56.27 %, H, 6.11 %; $[M+Na]^+$ *m/z* 321.095. $C_{14}H_{18}O_7$ requires C, 56.37 %, H, 6.08 %; $C_{14}H_{18}O_7Na$ requires *m/z* 321.095.

$[\alpha]_D^{293K}$ = +1.81° (c = 0.0105 gml^{-1}, MeOH)

^1H NMR δ_H CD_3CN: 3.26-3.31 (2H, m, 2-H, 4-H), 3.33-3.41 (2H, m, 3-H, 5-H), 3.56-3.60 (1H, dd, 6-H, J = 12.0, 5.2 Hz), 3.70-3.74 (1H, dd, 6-H', J = 12.0, 2.5 Hz) 3.74 (2H, s, $C\underline{H}_2Ph$), 5.45-4.47 (1H, d, 1-H, J = 8.1 Hz), 7.29-7.37 (5H, m, ArH).

^{13}C NMR δ_C CD_3CN: 40.0, 61.0, 69.5, 72.2, 75.9, 76.8, 94.1, 126.8, 128.2, 129.2, 133.6, 170.2. *m/z* (ES +ve ion mode) $[M+Na]^+$ 100 %.

1-([(2S)-phenyl]propanoyl)-β-D-glucopyranose (3.20)



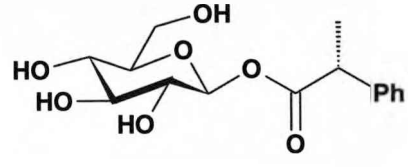
Yield: 88 %

Found: $[M+Na]^+$ *m/z* 335.111; $C_{15}H_{20}O_7Na$ requires *m/z* 335.1107.

^1H NMR δ_H d_6-acetone: 1.48-1.49 (3H, d, CH($C\underline{H}_3$), J = 7.15 Hz), 3.36-3.41 (2H, m, 2-H, 3-H), 3.43-3.48 (1H, m, 5-H), 3.52-3.57 (1H, t, 4-H, J = 8.95 Hz), 3.62-3.66 (1H, dd, 6-H, J = 12.1, 5.2 Hz), 3.78-3.81 (1H, dd, 6-H', J = 12.1, 2.3 Hz), 3.88-3.92 (1H, q, $C\underline{H}(CH_3)$, J = 7.1 Hz), 5.51-5.53 (1H, d, 1-H, J = 8.1 Hz), 7.27-7.39 (5H, m, ArH).

^{13}C NMR δ_C d_6-acetone: 19.7, 46.3, 62.3, 70.9, 73.7, 77.6, 78.6, 96.0, 128.4, 128.9, 129.9, 141.6, 174.9.

1-([(2R)-phenyl]propanoyl)-β-D-glucopyranose (3.19)



Yield: 90 %

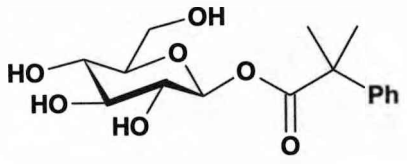
Found: $[M+Na]^+$ *m/z* 335.1095; $C_{15}H_{20}O_7Na$ requires *m/z* 335.1107.

$[\alpha]_D^{293K}$ = -7.18° (c = 0.017 gml^{-1}, MeOH)

^1H NMR δ_H d_6-acetone: 1.47-1.49 (3H, d, CH($C\underline{H}_3$), J = 7.2 Hz), 3.32-3.36 (1H, t, 2-H, J = 8.6 Hz), 3.44-3.53 (3H, m, 3-, 4-, 5-H), 3.69-3.73 (1H, dd, 6-H, J = 11.8, 4.4 Hz), 3.80-3.86 (2H, m, 6-H, $C\underline{H}(CH_3)$), 5.51-5.53 (1H, d, 1-H, J = 8.2 Hz), 7.25-7.30 (5H, m, Ar$\underline{H}$).

^{13}C NMR δ_C d_6-acetone: 19.7, 46.4, 62.8, 71.5, 74.2, 78.4, 78.8, 96.0, 128.2, 128.9, 129.7, 141.8, 174.1. *m/z* (ES +ve ion mode) 335 $[M+Na]^+$ 100 %

1-(2,2-dimethylphenyl)acetyl-β-D-glucopyranose (3.21)



Yield: 89 %

Found C, 58.71 %, H, 6.86 %; *m/z* $[M+Na]^+$ 349.1266; $C_{16}H_{22}O_7$ requires C, 58.89 %, H 6.79 %, $C_{16}H_{22}O_7Na$ requires *m/z* 349.1263.

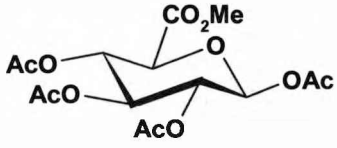
$[\alpha]_D^{293K}$ = +9.17° (c = 0.006 gml^{-1}, MeOH)

^1H NMR δ_H d_6-Acetone: 1.55 (3H, s, C(CH_3)), 1.59 (3H, s, C(CH_3)), 3.29-3.34 (1H, m, 2-H), 3.38-3.42 (2H, m, 4-H, 5-H), 3.48-3.52 (1H, m, 3-H), 3.67-3.72 (1H, m, 6-H), 3.80-3.83 (1H, m, 6'-H), 5.54-5.56 (1H, d, 1-H, J = 8.1 Hz), 7.21-7.25 (1H, m, ArH), 7.30-7.35 (2H, m, ArH), 7.39-7.42 (2H, m, ArH).

^{13}C NMR δ_C d_6-Acetone: 27.2, 27.8, 47.9, 63.0, 71.7, 74.2, 78.4, 78.8, 96.3, 127.1, 127.8, 129.5, 146.0, 176.2. *m/z* (ES +ve ion node) 349 $[M+Na]^+$ 100 %.

5.3 Synthesis of N-Glucuronides and Glucosides

Methyl 1,2,3,4-tetra-O-acetyl-β-D-glucopyranuronate[7]

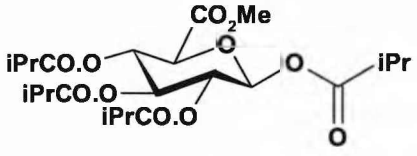


A solution of Glucurono-3,6-lactone (2 g, 11.36 mmol) in MeOH (28 ml) was evacuated with N_2. Triethylamine (0.06 ml) was added to the reaction which was left for 1h. Acetic acid (0.02 ml) was then added to quench the reaction and left stirring for 30 mins. The methanol was then removed *in vacuo*. The product was then dissolved in pyridine (5.66 ml) and cooled to 0^0C. The acetic anhydride (8.5 ml) was added drop wise to the reaction mixture (~15mins for addition) and the reaction left stirring for 3 hrs. The pyridine was removed *in vacuo*. Over ice, EtOAc (100 ml) and water (100 ml) was added to the residue followed by saturated $NaHCO_3$ (6 ml) until the solution was at neutral pH. The Organic layers were then separated and the aqueous layer extracted with EtOAc (2 x 60 ml). The organic layers were combined and dried using $MgSO_4$, and solvent removed *in vacuo*. A re-crystallisation was carried out using hot EtOAc and cold Hexane.

Yield: 78 %

^{1}H NMR δ_H d_6-Acetone: 1.98-2.10 (6H, 2xOAc), 2.07-2.10 (6H, 2xOAc), 3.71 (3H, s, CH_3), 4.51-4.56 (1H, d, 5-H), 5.03-5.14 (1H, dd, 2-H), 5.12-5.22 (1H, t, 3-H), 5.42-5.53 (1H, t, 4-H), 5.94-5.99 (1H, d, 1-H, J = 8.1 Hz)

Methyl (1,2,3,4-tetra-O-isobutyryl)-β-D-glucopyranuronate[8]



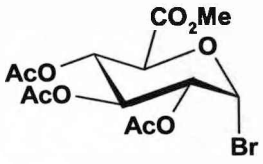
To a solution of Glucurono-3,6-lactone (22.2g, 0.12 mol) in MeOH (140 ml) was added triethylamine (0.4 ml) at RT. The reaction was then left for 4 hrs at which point the glucuronolactone had dissolved. The reaction was then neutralised with acetic acid (0.4 ml) and left stirring for a further 30 mins. The methanol was removed *in vacuo* and the residue dissolved in pyridine (60 ml) and DCM (60 ml). A solution of Isobutyryl chloride (100 ml) and DCM (100 ml) was added to the stirring reaction mixture dropwise via a pressure equalising dropping funnel at RT. After addition was complete the reaction was heated to 40°C for 3 hrs, and then allowed to cool to RT. The reaction was diluted with diethylether (150 ml) and water (150 ml) and the organic layer separated. The aqueous layer was extracted further with diethylether (2 x 150 ml) and the organic fractions combined. The combined organic extracts were successively washed with 3N HCl (100 ml), sat. $NaHCO_3$ (100 ml), brine (100 ml), water (100 ml) and then dried over $MgSO_4$. The solvent was removed and a re-crystallisation carried out using hexane.

Yield: 59 %. Mp: 127-128°C

^{1}H NMR δ_H $CDCl_3$: 1.13-1.17 (24H, m, $CH(C\underline{H}_3)_2$), 2.45-2.63 (4H, m $C\underline{H}(CH_3)_2$), 3.73 (2H, s, $CO_2C\underline{H}_3$), 4.18-4.20 (1H, d, 5-H, J = 9.9 Hz), 5.21-5.27 (2H, m, 2-H, 4-H), 5.37-5.42 (1H, t, 3-H, J = 9.5 Hz), 5.77-5.79 (1H, d, 1-H, J = 8.2 Hz)

^{13}C NMR δ_C $CDCl_3$: 18.6, 19.1, 19.2, 19.3, 34.1, 34.2, 34.3, 34.5, 53.2, 69.2, 70.0, 71.8, 73.6, 91.8, 167.1, 175.3, 175.4, 175.7, 176.1.

Methyl (2,3,4-tri-O-acetyl)-α-D-glucopyranuronate bromide (1.6)[7]

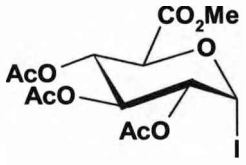


To a solution of starting material (1 g, 2.52 mmol) in DCM (6 ml) at 0^0C was added drop wise, HBr in AcOH (45 w/v %) (5.3 ml, 27.7 mmol). The reaction was then allowed to reach RT after addition and left stirring for 3hrs. The acid was then quenched with saturated $NaHCO_3$ (30 ml) and the organic layer separated. The Aqueous layer was then extracted further with DCM (3 X 50 ml). The organic layers were combined and washed with water (2 x 40 ml). The DCM was then dried over $MgSO_4$, and evaporated to dryness. A re-crystallisation was then carried out using diethyl ether which gave a white crystalline solid

52% yield

^{1}H NMR δ_H $CDCl_3$: 2.01-2.13 (9H, 3xs, 3xOAc), 3.72 (3H, s, CO_2CH_3), 4.54-4.61 (1H, d, 5-H, J = 10.3 Hz), 4.80-4.91 (1H, dd, 2-H), 5.18-5.32 (1H, t, 4-H, J = 10.2 Hz), 5.56-5.69 (1H, t, 3-H, J = 9.6 Hz), 6.62-6.69 (1H, d, 1-H, J = 4.0 Hz).

Methyl (2,3,4-tri-O-acetyl)-α-D-glucopyranuronate iodide (4.53)



A solution of starting material (500 mg, 1.33 mmol) in acetonitrile (2 ml) was evacuated with N_2. TMSI (0.28 ml, 2 mmol) was added to the stirring solution and the reaction heated to 50^0C for 2 h. The reaction was then allowed to cool and acetonitrile removed *in vacuo*. The residue was re-dissolved in ethyl acetate (15 ml) and extracted with $Na_2S_2O_3$ (10 % w/v) (15 ml) followed by $NaHCO_3$ (10 ml) and water (10 ml). The ethyl acetate layer was extracted further with water (2 x 10 ml). The organic layer was then dried over $MgSO_4$ and evaporated to dryness. The residue was then re-crystallised from ethanol[9].

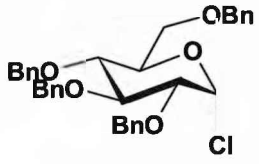
Yield: 85%

Mp: 103-105°C. Found: $C_{13}H_{17}O_9INa$ $[M+Na]^+$ *m/z* 466.9815 requires 466.98089

^{1}H NMR δ_H $CDCl_3$ 2.01-2.15 (9H, 3x s, 3xOAc), 3.77 (3H, s, OCH_3), 3.20-3.25 (1H, dd, 2-H, J = 9.8, 4.4 Hz), 4.31-4.39 (1H, d, 5-H, J = 10.2 Hz), 5.20-5.29 (1H, t, 4-H, J = 9.5 Hz), 5.49-5.58 (1H, t, 3-H, J = 9.7 Hz), 7.0-7.04 (1H, d, 1-H, J = 4.3Hz).

^{13}C NMR δ_C $CDCl_3$: 20.8, 20.9, 21.1, 53.4, 68.6, 70.4, 71.3, 71.4, 75.1, 166.9, 169.7, 169.8, 169.9. *m/z* (ES +ve ion mode) 467 $[M+Na]^+$ 100 %.

2,3,4,6-(tetra-O-benzyl)-α-D-glucopyranose chloride (4.86)



To a solution of tetra-*O*-benzyl glucopyranose (579 mg, 1.07 mmol) in DCE (6 ml) was added $SOCl_2$ (1 ml, 13.7 mmol) and DMF (0.05 ml). The reaction was left stirring at RT for 17 hrs. The reaction was then quenched over ice carefully with water (~10 ml drop wise), then sat.$NaHCO_3$ (~5 ml) until neutralised. The solution was then diluted with DCM (30 ml) and the organic layer collected. The organic layer was then washed with brine (20 ml) and water (20 ml) and dried over Na_2SO_4. The solvent was removed *in vacuo* to give the product as a viscous oil.[10,11]

Yield: 98 %

Found: $[M+Na]^+$ *m/z* 581.207 $C_{34}H_{35}O_5{}^{35}ClNa$ requires *m/z* 581.202

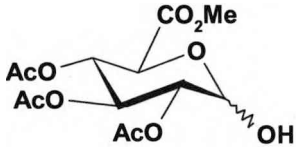
^1H NMR δ_H $CDCl_3$: 3.51-3.57 (1H, dd, C<u>H</u>$_2$OBn), 3.61-3.71 (3H, m, C<u>H</u>$_2$OBn, 2-H, 4-H), 3.92-4.03 (2H, m, 3-H, 5-H), 4.32-4.50 (3H, 3 x d, 3 x C<u>H</u>$_2$Ar), 4.61 (2H, s, $CH_2OC\underline{H}_2$-Ar), 4.70-4.78 (2H, 2 x d, C<u>H</u>$_2$Ar), 4.85-4.91 (1H, d, C<u>H</u>$_2$Ar), 5.97-6.0 (1H, d, 1-H, J = 3.7 Hz), 7.02-7.06 (2H, m, Ar<u>H</u>), 7.15-7.28 (18H, m, Ar<u>H</u>).

^{13}C NMR δ_C $CDCl_3$: 68.2, 73.4, 73.8, 73.9, 75.7, 76.3, 76.8, 80.2, 81.8, 94.0, 128.2, 128.3, 128.4, 128.4, 128.5, 128.5, 128.6, 129.0, 137.9, 138.1, 138.5, 138.9.

-General procedure for the selective deprotection of the anomeric ester

To a solution of anomeric ester protected sugar (5.13 mmol) in DMF (15 ml) at -10°C was added AcOH (0.43 ml, 7.18 mmol). Hydrazine monohydrate (0.34 ml, 7.18 mmol) was then added to the reaction mixture which was kept at -10°C for 4 hrs. The reaction was then diluted with DCM (50 ml) and then neutralised with sat. $NaHCO_3$ (~ 10 ml) at 0°C. The organic layer was then separated and the aqueous layer extracted with DCM (3 x 30 ml). The organic layers were then combined and washed with brine (100 ml), and water (3 x 50 ml). The DCM was then dried over Na_2SO_4 and evaporated to dryness. The residue was purified by column chromatography, eluting with 30-70 % EtOAc/hexane.

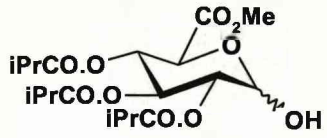
Methyl (2,3,4-tetra-O-acetyl)-α,β-D-glucopyranuronate (4.61)[12]



^{1}H NMR δ_H $CDCl_3$ α/β ratio 8:1: 2.0-2.12 (18H, 6 x s, α and β OAc), 3.75 (3H, s, α-CO_2-CH_3), 3.76 (3H, s, β-CO_2CH_3), 4.13-4.16 (1H, m, β 5-H), 4.58-4.61 (1H, d, 5-H, J = 10.6 Hz), 4.88-4.95 (2H, m, α-2-H, β-1-H, J = 7.8 Hz), 5.15-5.32 (4H, m, α-4-H, β-2-H, 3-H, 4-H), 5.54-5.60 (2H, d and t, 1-H, J = 3.9 Hz, 3-H, J = 9.5 Hz).

^{13}C NMR δ_C $CDCl_3$: 20.9, 20.9, 20.9, 21.1, 21.4, 53.3, 53.4, 60.9, 68.4, 69.5, 69.8, 69.9, 71.1, 71.9, 72.9, 73.2, 90.6, 95.8, 167.9, 168.8, 169.9, 170.1, 170.4, 170.5, 170.9, 171.8.

Methyl (2,3,4-tri-O-isobutyryl)-α,β-D-glucopyranuronate (4.129)[8]



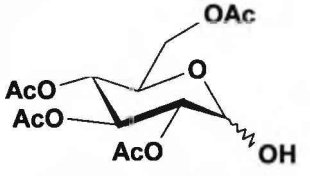
Column chromatography carried out using 30-45 % EtOAc/Hexane

Yield: 74 % 0.3:1 β/α ratio

^{1}H NMR δ_H $CDCl_3$: 1.08-1.16 (18H, m, $CH(C\underline{H}_3)_2$), 2.47-2.58 (3H, m, $C\underline{H}(CH_3)_2$), 3.57-3.58 (1H, dd, α-OH), 3.72 (3H, s, $\alpha CO_2C\underline{H}_3$) 3.74 (3H, s, $\beta CO_2C\underline{H}_3$), 4.10-4.13 (1H, d, β5-H, J = 9.9 Hz), 4.59-4.61 (1H, d, α5-H, J = 10.2 Hz), 4.77-4.81 (1H, dd, β1-H, J = 8.3 Hz), 4.90-4.97 (2H, m, α2-H, β2-H), 5.17-5.26 (2H, m, α4-H, β4-H), 5.36-5.41 (1H, t, β3-H, J = 9.6 Hz), 5.54-5.56 (1H, dd, α1-H, J = 3.8 Hz), 5.62-5.67 (1H, t, α3-H, J = 9.9 Hz)

^{13}C NMR δ_C $CDCl_3$: 19.0, 19.1, 19.2 (x 2), 19.3, 19.3, 34.1, 34.2, 34.3, 34.4, 53.1, 53.3, 68.5, 69.0, 69.5, 69.6, 70.9, 71.3, 73.1, 73.2, 90.7, 96.1, 167.8, 168.8, 175.8, 175.9, 176.1, 176.5, 177.3.

2,3,4,6-(tetra-O-acetyl)-α,β-D-glucopyranose (4.73)[13]

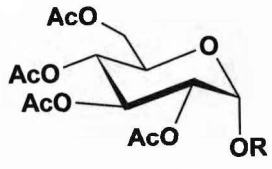


Found: $[M+Na]^+$ *m/z* 371.095 $C_{14}H_{20}O_{10}Na$ requires *m/z* 371.095

^{1}H NMR δ_H $CDCl_3$: 2.01-2.03 (12H, s, α and β 4 x OAc), 2.09-2.10 (12H, s, α and β 4 x OAc), 3.73-3.77 (1H, m, β5-H), 4.11-4.17 (2H, β6-H, α6-H), 4.22-4.29 (3H, β6'-H, α6'-H, α5-H), 4.73-4.75 (1H, d, β1-H, J = 8.0 Hz), 4.88-4.92 (2H, m, α2-H, β2-H), 5.05-5.08 (2H, 2 x t, α4-H, β4-H), 5.10-5.25 (2H, t, β3-H), 5.46-5.47 (1H, d, α1-H, J = 3.6 Hz), 5.51-5.56 (1H, t, α3-H).

^{13}C NMR δ_C $CDCl_3$: 20.9, 20.9, 21.0, 21.0, 21.1, 62.4, 67.7, 68.9, 68.9, 70.3, 71.5, 72.5, 72.6, 73.7, 77.1, 90.6, 95.9, 170.0, 170.5, 170.5, 171.2, 171.2. *m/z* (ES +ve ion mode) $[M+Na]^+$ 371 100 %.

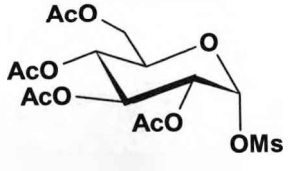
-General synthesis of anomeric sulfonate sugars



R = Ms or Ts

To a solution of starting material (0.57 mmol) and DCM (3 ml) was added collidine (0.22ml, 1.72 mmol) at RT under N_2. The sulfonic anhydride (0.86 mmol) was added to the reaction mixture as a solution in DCM (1 ml). The reaction was left stirring for 3 h. The reaction was then diluted with DCM (15 ml) and successively washed with 1M HCl (20 ml), 1M $NaHCO_3$ (20 ml) and water (2 x 20 ml). The DCM was then dried over $MgSO_4$ and evaporated to dryness. Column chromatography was carried out using EtOAc/Hex (60:40).

2,3,4,6-Tetra-O-acetyl-1-O-(methylsulfonyl)-α-D-glucopyranose (4.76)[14]



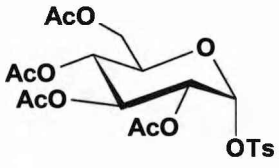
Yield: 29%

Mp: 109-112°C. Lit. 108-110°C[14]. Found: $[M+Na]^+$ *m/z* 449.0714 $C_{15}H_{22}O_{12}SNa$ requires *m/z* 449.0730

^1H NMR δ_H $CDCl_3$: 2.0-2.11 (12H, 4 x s, 4 x CCH_3), 3.09 (3H, s, OSO_2CH_3), 4.09-4.32 (3H, m, 5-H, CH_2), 5.01-5.08 (1H, dd, 2-H, J = 10.3, 3.7 Hz), 5.10-5.18 (1H, t, 4-H, J = 9.6 Hz), 5.44-5.51 (1H, t, 3-H, J = 10.1 Hz), 6.06-6.08 (1H, d, 1-H, J = 3.7 Hz)

^{13}C NMR δ_C $CDCl_3$: 20.8, 20.8, 20.9, 20.9, 39.8, 61.8, 68.0, 69.4, 69.5, 70.6, 96.3, 169.7, 170.1, 170.2, 170.7. *m/z* (ES +ve ion mode) 449 $[M+Na]^+$ 100 %.

2,3,4,6-Tetra-O-acetyl-1-O-(toluenesulfonyl)-α-D-glucopyranose (4.78)[14]



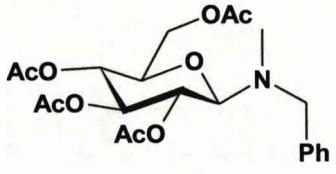
Yield: 22 %

Found: $[M+Na]^+$ *m/z* 525.1055 $C_{21}H_{26}O_{12}SNa$ requires *m/z* 525.1043

^1H NMR δ_H $CDCl_3$ **250 MHz:** 1.96 (3H, s, OAc), 2.01 (6H, s, 2x OAc), 2.05 (3H, s, OAc), 2.46 (3H, s, $ArCH_3$), 3.65-3.73 (1H, dd, 6-H, J = 12.7, 2.2 Hz) 3.87-3.96 (1H, m, 5-H) 4.08-4.16 (1H, dd, 6-H', J = 12.5, 3.6 Hz), 4.92-4.99 (1H, dd, 2-H, J = 10.3, 3.6 Hz), 5.05-5.14 (1H, t, 4-H, J = 9.4 Hz), 5.39-5.49 (1H, t, 3-H, J = 9.4 Hz), 6.01-6.04 (1H, d, 1-H, J = 3.6 Hz).

Reactions of secondary and primary amines with the anomeric bromo sugar

2,3,4,6-Tetra-O-(acetyl)-1-N-methylbenzyl-β-glucopyranose (4.62)[15]



2,3,4,6-Tetra-*O*-acetyl glucopyranosyl bromide (616mg, 1.5 mmol) and benzyl-*N*-methylamine (0.36 ml, 3 mmol) were warmed together at 35°C in diethylether (5 ml) until most of the ether had evaporated to a 1ml concentration. The mixture was then left at RT for 1.5 hrs, with the HBr salt of benzyl-*N*-methylamine beginning to precipitate on cooling. The reaction was then filtered and washed through with diethylether. The filtrate was then extracted with 10 % w/v aq citric acid (2 x 10 ml) and the ether dried over Na_2SO_4, filtered and evaporated to dryness. A re-crystallisation was carried out using iso-hexane and a few drops of ether on cooling to bring on crystallisation.[15]

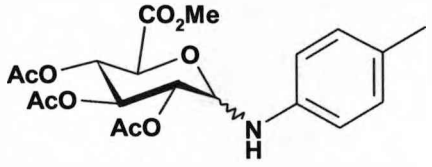
Yield: 29 %

Found: $C_{22}H_{30}O_9N$ $[M+H]^+$ *m/z* 452.1917

^1H NMR δ_H $CDCl_3$: 1.99 (3H, s, OAc), 2.01 (3H, s, OAc), 2.06 (3H, s, OAc), 2.10 (3H, s, OAc), 2.37 (3H, s, NCH_3), 3.53-3.58 (1H, m, 5-H), 3.74-3.79 (1H, d, CH_2Ar, J = 13.8 Hz), 3.85-3.89 (1H, d, CH_2Ar, J = 13.8 Hz), 4.04-4.11 (1H, d, 1-H, J = 8.8 Hz), 4.13-4.17 (1H, dd, 6'-H, J = 12.0, 2.5 Hz), 4.22-4.26 (1H, dd, 6-H, J = 12.3, 5.0 Hz), 4.99-5.04 (1H, t, 4-H, J = 9.5 Hz), 5.13-5.18 (1H, t, 2-H, 9.3 Hz), 5.19-5.24 (1H, t, 3-H, J = 9.0 Hz), 7.22-7.32 (5H, m, ArH).

^{13}C NMR δ_C $CDCl_3$: 20.6, 20.6, 20.7, 35.2, 57.9, 61.0, 62.4, 66.4, 68.2, 69.0, 72.9, 74.1, 91.9, 127.2, 128.3, 128.5, 138.7, 139.3, 169.5, 169.5, 170.2, 170.6. *m/z* (ES +ve ion mode) 452 $[M+H]^+$ 100 %.

Methyl-(2,3,4-Tri-O-acetyl)-1-(4-methylphenyl)amino-α,β-glucopyranuronate (4.60)



To a solution of bromo-sugar **(1.6)** (250 mg, 0.63 mmol) in acetone (3 ml) under nitrogen was added *p*-toluidine (50 mg, 0.47 mmol) in acetone (1 ml); the solution turned brown on addition. Then 10 % w/v NaOH (0.25 ml, 0.63 mmol) was added to the reaction mixture and the brown colour intensified. The reaction was left stirring at room temperature for 5 days. The solvent was then removed *in vacuo* and the oily residue taken up in DCM (10 ml). The DCM was extracted with water (2 x 30 ml) and the DCM dried over $MgSO_4$. Column chromatography was carried out using 40-50 % EtOAc/Hexane. The fractions containing product were collected and solvent removed, a recrystallisation was carried out using diethylether/hexane which gave a white crystalline solid.

Yield: 71 %

Found: $C_{20}H_{25}NO_9Na$ $[M+Na]^+$ *m/z* 446.1427, requires *m/z* 446.1440

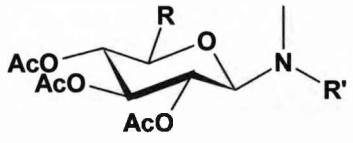
1:3 α/β Ratio, ^{1}H NMR δ_H β-anomer d_6-Acetone: 1.96 (3H, s, OAc), 1.97(3H, s, OAc), 1.98 (3H, s OAc), 2.19 (3H, s, $ArC\underline{H}_3$), 3.65 (3H, s, $CO_2C\underline{H}_3$), 4.43-4.46 (1H, d, 5-H, J = 10.1 Hz), 5.03-5.06 (1H, d, 1-H, J = 8.5 Hz), 5.10-5.15 (1H, t, 4-H, J = 9.3 Hz), 5.20-5.26 (1H, dd, 2-H, J = 9.1 Hz), 5.40-5.46 (1H, t, 3-H, J = 9.5 Hz), 6.71-6.75 (1H, m, ArH), 6.87-6.91 (1H, m, ArH), 6.95-7.00 (2H, m, ArH).

^{1}H NMR δ_H α-anomer d_6-Acetone: 2.01 (3H, s, OAc), 2.02 (3H, s, OAc), 2.05 (3H, s, OAc), 2.21 (3H, s, $ArC\underline{H}_3$), 3.70 (3H, s, $CO_2C\underline{H}_3$), 4.45-4.47 (1H, d, 5-H, J = 10 Hz), 4.99-5.04 (1H, t 2-H, J = 8 Hz), 5.14-5.19 (1H, t, 3-H, 8 Hz), 5.46-5.50 (1H, t, 4-H, J = 8.5 Hz), 5.55-5.59 (1H, dd, 1-H, J = 7.0, 4.5 Hz), 5.89-5.94 (1H, d, N$\underline{H}$, J = 7.0 Hz), 6.71-6.75 (1H, m, ArH), 6.87-6.91 (1H, m, ArH), 6.95-7.00 (2H, m, ArH)

^{13}C NMR δ_C d_6-Acetone (α and β): 20.7, 20.8, 20.8, 20.9, 21.0, 29.6, 53.0, 53.1, 69.9, 70.1, 70.4, 70.4, 70.5, 71.2, 72.2, 73.6, 73.9, 74.1, 80.2, 84.8, 115.6, 115.7, 129.2,

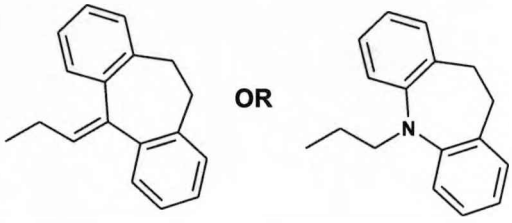
129.2, 130.7, 144.4, 144.5, 144.5, 168.8, 169.7, 170.2, 170.2, 170.3, 170.6, 170.6, 170.8. *m/z* (ES +ve ion mode) 446 $[M+Na]^+$ 100 %.

- Reaction of the anomeric bromo sugar with drug like amines



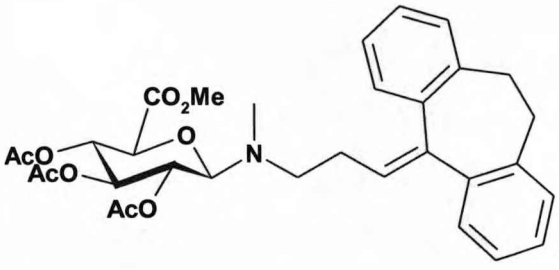
R= CH_2OAc, CO_2Me

R'=



To a solution of bromosugar **(1.6)** or **(1.20)** (0.75 mmol) in acetone (6 ml) was added the secondary amine (1.5 mmol) in a solution of DCM (6 ml), followed by 10 % w/v sodium carbonate (0.75 mmol). The reaction was heated with stirring to 45^0C for 4 hrs without a condenser to allow some solvent to evaporate. The remaining solvent was then removed *in vacuo*. Column chromatography was then carried out using 10-30 % EtOAc/Isohexane.

N-(Methyl (2,3,4-tri-O-acetyl)-β-D-glucopyranuronate)-N-methyl-3-(10,11-dihydro-5H-dibenzo[a,d]cycloheptan-5-ylidene)-1-propanamine (4.67)



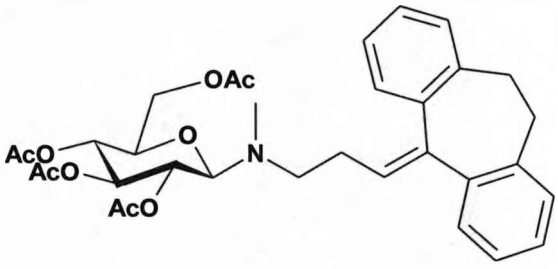
Yield: 17%

Found: C, 66.70, H, 6.77, N, 2.15; [M+H] *m/z* 580.254. $C_{32}H_{38}O_9N$ requires C, 66.31, H, 6.43, N, 2.42 % $C_{32}H_{39}O_9N$ requires *m/z*, 580.256

^{1}H NMR δ_H $CDCl_3$: 1.74 (3H, s, OAc), 1.98-2.01 (6H, 2s, OAc), 2.2-2.28 (2H, broad m, $CH_2C\underline{H}_2CH$), 2.29 (3H, s, $NC\underline{H}_3$), 2.65-2.74 (1H, m, $NC\underline{H}_2$), 2.75-2.88 (2H, broad s, $ArC\underline{H}_2CH_2$, m, $NC\underline{H}_2$), 2.90-3.02 (1H, broad s, $CH_2C\underline{H}_2Ar$), 3.22-3.47 (2H, broad s, $ArC\underline{H}_2C\underline{H}_2Ar$), 3.72 (3H, s, $CO_2C\underline{H}_3$), 3.78-3.90 (1H, broad s, 5-H), 4.05-4.13 (1H, s, 1-H), 5.07-5.17 (2H, t, 4-H, J = 9.5 Hz, broad peak, 2-H), 5.17-5.24 (1H, t, 3-H, J = 9.3 Hz), 5.76-5.82 (1H, t, $CH_2C\underline{H}$, J = 7.3 Hz), 6.99-7.04 (1H, ArH), 7.09-7.27 (7H, ArH).

^{13}C NMR δ_C $CDCl_3$: 20.3, 20.5, 20.6, 28.0, 32.0, 33.8, 34.4, 35.3, 52.6, 53.2, 53.9, 67.7, 69.9, 73.3, 73.9, 125.6, 125.9, 127.0, 127.4, 128.0, 128.2, 128.5, 129.9, 137.1, 139.4, 140.0, 141.3, 143.6, 167.7, 169.3, 170.0, 171.1. *m/z* (ES +ve ion mode) 580 $[M+H]^+$ 100 %

N(2,3,4,6-Tetra-(O-acetyl)-β-D-glucopyranosyl)-N-methyl-3-(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-ylidene)-1-propanamine (4.68)



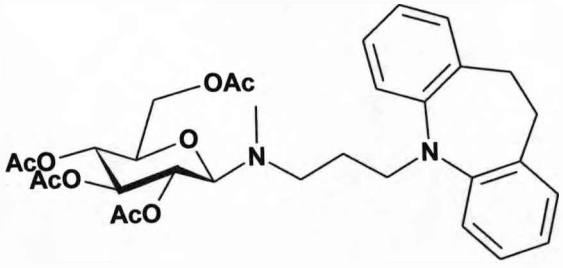
Yield: 29%

Found [M+H] *m/z* 594.27002 $C_{33}H_{40}O_9N$ requires *m/z* 594.269

^{1}H NMR δ_H $CDCl_3$: 1.76 (3H, s, OAc), 1.98 (3H, s, OAc), 2.01 (3H, s, OAc), 2.05 (3H, s, OAc), 2.21-2.27 (2H, broad s, $CHCH_2$), 2.25-2.29 (3H, s, NCH_3), 2.64-2.72 (1H, m, NCH_2), 2.73-2.84 (1H, broad s, $ArCH_2CH_2$), 2.76-2.85 (1H, m, NCH_2), 2.88-3.02 (1H, broad s, $ArCH_2CH_2$), 3.22-3.55 (2H, 2 x broad s, $ArCH_2CH_2Ar$), 3.45-3.50 (1H, broad s, 5-H), 4.01-4.07 (2H, dd, 6-H', J = 12.0, 2.3 Hz, underneath 6-H' broad s 1-H), 4.16-4.21 (1H, dd, 6-H, J = 12.0, 4.8 Hz), 4.91-4.99 (1H, t, 4-H, J = 9.5 Hz), 5.07-5.19 (2H, broad m, 2-H, t, 3-H, J = 9.3 Hz), 5.77-5.83 (1H, t, CH_2CH, J = 7.5 Hz), 6.99-7.04 (1H, m, ArH), 7.09-7.27 (7H, m, ArH)

^{13}C NMR δ_C $CDCl_3$: 20.4, 20.6, 20.7, 28.0, 32.1, 33.8, 60.3, 62.4, 68.1, 68.9, 72.9, 74.1, 125.7, 126.0, 127.0, 127.4, 128.1, 128.3, 128.5, 129.3, 129.9, 137.1, 139.4, 140.1, 141.3, 143.6, 169.5, 170.2, 170.7. *m/z* (ES +ve ion mode) 594 $[M+H]^+$ 100 %

N(2,3,4,6-Tetra-(O-acetyl)-β-D-glucopyranosyl)-N-methyl-3-(10,11-dihydro-5H-dibenzo[b,f]azepin-5-yl)-1-propanamine (4.69)



Yield: 16 %. After column chromatography the material required further purification using preparative HPLC to remove the E2 product (see experimental introduction for specifications). The column was eluted with an increased gradient of MeCN/H_2O under neutral conditions.

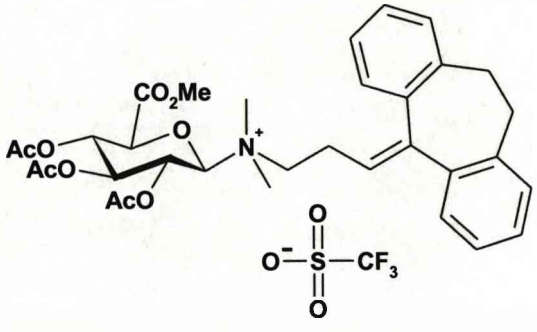
Found: $[M+H]^+$ *m/z*, 597.281, $C_{32}H_{41}O_9N_2$ requires *m/z* 597.285

^1H NMR δ_H $CDCl_3$: 1.64-1.72 (2H, m, $CH_2C\underline{H}_2CH_2$), 1.86 (3H, s, OAc), 1.99 (3H, s, OAc), 2.01 (3H, s, OAc), 2.05 (3H, s, OAc), 2.28 (3H, s, $NC\underline{H}_3$), 2.64-2.76 (2H, m, $NC\underline{H}_2CH_2$-CH_2), 3.16 (4H, s, $C\underline{H}_2C\underline{H}_2$), 3.44-3.50 (1H, m, 5-H), 3.69-3.73 (2H, t, $CH_2CH_2C\underline{H}_2$, J = 6.8 Hz), 3.99-4.01 (1H, d, 1-H, J = 9.0 Hz), 4.02-4.06 (1H, dd, 6-H', J = 12.3, 2.5 Hz), 4.15-4.20 (1H, dd, 6-H, J = 12.1, 4.8 Hz), 4.93-4.98 (1H, t, 4-H, J = 9.3 Hz), 5.05-5.10 (1H, t, 2-H, J = 9.3 Hz), 5.12-5.17 (1H, t, 3-H, J = 9.3 Hz), 6.88-6.93 (2H, m, ArH), 7.03-7.13 (4H, m, ArH).

^{13}C NMR δ_C $CDCl_3$: 20.5, 20.6, 20.7, 26.3, 32.2, 34.7, 48.0, 51.8, 62.4, 68.2, 68.9, 72.8, 74.1, 91.6, 93.1, 119.9, 122.5, 126.4, 129.8, 134.2, 148.4, 169.5, 170.7. *m/z* (ES, +ve ion mode) 597 $[M+H]^+$ 100 %

Method for the quaternisation using methyl triflate

N-(Methyl-(2,3,4-tri-O-acetyl)-β-D-glucopyranuronate)-N,N-dimethyl-3-(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-ylidene)-1-propanammonium trifluoromethane sulfonate (4.75)



To a solution of starting material **(4.67)** (35 mg, 0.06 mmol) in DCM (4 ml) under nitrogen, was added methyltriflate (0.014 ml, 0.12 mmol) at room temperature. The solution was left stirring for 2 h. The DCM was removed *in vacuo* and column chromatography used to purify, the eluting solvent being 10% IPA/DCM.

Yield: 21 %

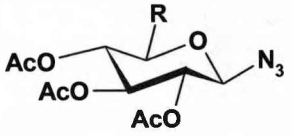
Found: [M+H] *m/z* 594.2718 $C_{33}H_{40}O_9N$ requires 594.270

^{1}H NMR δ_H d_6DMSO and d_4-acetic acid @373K: 1.99 (3H, s, OAc), 2.08 (3H, s, OAc), 2.15 (3H, s, OAc), 2.61-2.78 (2H, m, $C\underline{H}_2CH$), 3.00-3.12 (4H, m, $C\underline{H}_2C\underline{H}_2$), 3.12 (3H, s, $NC\underline{H}_3$), 3.18 (3H, s, $NC\underline{H}_3$), 3.63-3.69 (2H, m, $NC\underline{H}_2$), 3.79 (3H, s, $CO_2C\underline{H}_3$), 5.1 (1H, s, 5-H), 5.19-5.25 (4H, m, 1-H, 2-H, 3-H, 4-H), 5.75-5.79 (1H, t, $CH_2C\underline{H}$), 7.06-7.28 (8H, m, ArH)

^{13}C NMR δ_C d_6-DMSO: 19.9, 20.1, 20.4, 22.7, 31.1, 32.6, 46.9, 48.3, 52.5, 62.3, 63.2, 64.2, 64.7, 75.5, 86.7, 124.0, 125.5, 125.7, 127.1, 127.5, 127.6, 128.1, 129.5, 136.5, 138.4, 138.5, 140.0, 145.2, 166.9, 167.0, 168.2, 168.4. *m/z* (ES +ve ion mode) 594 $[M+H]^+$ 100 %.

The Azide Route

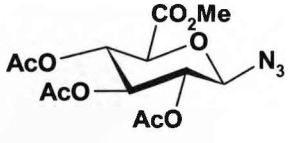
- General method for the preparation of the anomeric azide



R= CH_2OAc, CO_2Me

To a solution of 1-*O*-acetyl sugar (30 mmol) in DCM (20 ml) under a nitrogen atmosphere was added Tin (IV) Chloride (1.05 ml, 9 mmol), followed by trimethylsilyl azide (5.57 ml, 42 mmol). The reaction was left stirring at room temperature for 24 h. The reaction was then diluted with DCM (100 ml) and carefully quenched with saturated Na_2CO_3 (50 ml) at 0^0C. The Tin residues were then filtered off, and the filtrate collected and extracted with Na_2CO_3 (1 X 50 ml) and water (2 x 50 ml). The DCM layer was then dried over $MgSO_4$ and evaporated to dryness. The off white solid was then recrystalised from ethanol to give a crystalline white solid.

Methyl (2,3,4-tri-O-acetyl)-β-D-glucopyranuronate azide (4.90)[16]



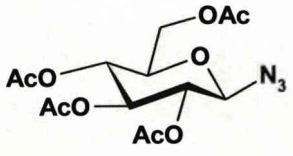
Yield: 67%

Mp: 151-152°C. Found: $[M+H]^+$ *m/z*, 382.0847; C, 43.22, H, 4.74, N, 11.38 %. $C_{13}H_{17}O_9N_3Na$ requires *m/z* 382.0862; $C_{13}H_{17}O_9N_3$ requires C, 43.47, H, 4.77, N, 11.70 %.

^{1}H NMR δ_H $CDCl_3$: 2.02-2.03 (6H, 2s, OAc), 2.07 (3H, s, OAc), 3.78 (3H, s, CO_2CH_3), 4.10-4.14 (1H, d, 5-H, J = 9.5 Hz), 4.69-4.73 (1H, d, 1-H, J = 8.7 Hz), 4.93-4.99 (1H, t, 3-H, J = 8.7 Hz), 5.21-5.31 (2H, m, 2-H, 4-H).

^{13}C NMR δ_C $CDCl_3$: 20.8, 20.9, 53.4, 69.4, 70.9, 72.3, 74.7, 88.5, 118.1, 166.9, 169.4, 169.9, 170.3

2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl azide (4.91)[17]



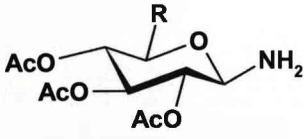
Yield: 98%

Mp: 128-129°C. lit: 127°C Found: $[M+NH_3]^+$ *m/z* 391.145; C, 44.91, H, 5.10, N, 11.30 %. $C_{14}H_{23}O_9N_4$ requires *m/z* 391.146. $C_{14}H_{19}O_9N_3$ requires C, 45.04, H, 5.13, N, 11.26 %.

^1H NMR δ_H $CDCl_3$: 2.01-2.03 (6H, 2s, OAc), 2.09-2.14 (6H 2s, OAc), 3.76-3.86 (1H, m, 5-H), 4.11-4.22 (1H, dd, 6-H', J = 12.4, 2.3 Hz), 4.25-4.34 (1H, dd, 6-H, J = 12.4, 4.8 Hz), 4.62-4.69 (1H, d, 1-H, J = 8.8 Hz), 4.91-5.01 (1H, t, 2-H, J = 9.3 Hz), 5.08-5.13 (1H, t, 4-H, J = 9.8 Hz), 5.19-5.24 (1H, t, 3-H, J = 9.5 Hz).

^{13}C NMR δ_C $CDCl_3$: 20.9, 21.0, 62.1, 68.3, 71.1, 73.0, 74.4, 88.3, 169.5, 169.6, 170.4, 170.9. *m/z* (CI +ve ion mode) $[M+NH_3]$ 391 100 %.

- General method for the reduction of the Azidosugar

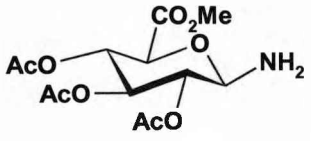


R= CH_2OAc, CO_2Me

To a solution of azidosugar **(4.90** or **4.91)** (7 mmol) in ethyl acetate (20 ml) was added Pd/C (1.48 g, 20 mol %). The residual Pd/C was washed down into the flask with methanol (20 ml), and a vacuum applied to the flask. A positive pressure of hydrogen was applied to the reaction which was magnetically stirred at room temperature for 4 hrs. The Pd/C was filtered onto cellite and ethyl acetate used to

wash the celite pad. The solvent was removed *in vacuo*. A re-crystallisation was carried out on using hot ethanol, on cooling addition of hexane or diethyl ether slowly brought on crystallisation.

Methyl-(2,3,4-tri-O-acetyl)-1-N-amino-β-D-glucopyranuronate (4.92)[18]



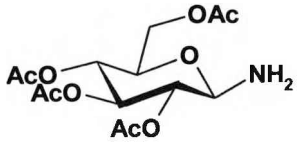
Yield: 35%

Mp: 140-142°C. Found: C, 46.63, H, 5.74 N, 4.17 %; $[M+H]^+$ *m/z* 334.1142. $C_{13}H_{19}O_9N$ requires C, 46.85, H, 5.74, N, 4.20 %; $C_{13}H_{20}O_9N$ requires *m/z* 334.1138

^1H NMR δ_H $CDCl_3$: 2.01-2.05 (6H, s, 2 x OAc), 2.08 (3H, s, OAc), 3.77 (3H, s, CO_2CH_3), 4.02-4.08 (1H, d, 5-H, J = 10.0 Hz), 4.21-4.25 (1H, d, 1-H, J = 9.0 Hz), 4.81-4.87 (1H, t, 2-H, J = 9.5 Hz), 5.11-5.22 (1H, t, 3-H, J = 9.5 Hz), 5.27-5.38 (1H, t, 4-H, J = 9.6 Hz).

^{13}C NMR δ_C $CDCl_3$: 20.8, 20.9, 21.0, 68.8, 70.3, 72.1, 72.8, 73.9, 85.7, 168.0, 169.8, 170.3, 170.4. *m/z* (Cl +ve ion mode) 334 $[M+H]^+$ 100 %.

(Tetra-2,3,4,6-O-acetyl)-1-N-amino-β-D-glucopyranuronate (4.93)[19]



Yield: 99%

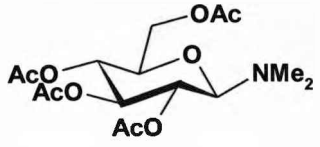
Mp: 161-162°C. Lit: 163-164°C[19]. Found $C_{14}H_{21}NO_9Na$ $[M+Na]^+$ *m/z* 370.1114 $C_{14}H_{21}NO_9Na$ requires 370.1128

^1H NMR δ_H $CDCl_3$: 2.01 (3H, s, OAc), 2.02 (3H, s, OAc), 2.07 (3H, s, OAc), 2.09 (3H, s, OAc), 3.66-3.72 (1H, m, 5-H), 4.09-4.14 (1H, dd, 6-H', J = 12.3, 2.3 Hz), 4.16-4.21 (1H, broad d, 1-H), 4.20-4.26 (1H, dd, 6-H, J = 12.3, 4.9 Hz), 4.79-4.86 (1H, t, 2-H, J = 9.6 Hz), 5.01-5.07 (1H, t, 3-H, 9.6 Hz), 5.21-5.27 (1H, t, 4-H, J = 9.6 Hz)

^{13}C NMR δ_C $CDCl_3$: 21.0, 21.1, 21.2, 62.7, 69.3, 72.5, 73.2, 73.6, 85.4, 118.1, 169.9, 170.5, 171.0. *m/z* (ES +ve ion mode) $[M+Na]^+$ 370 100 %.

N,N-Dimethylation of the aminosugar

2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl-N,N-dimethylamine (4.94)



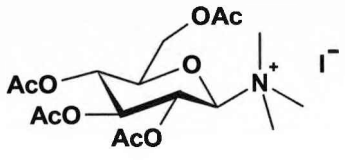
To a solution of **(4.93)** (400 mg, 1.15 mmol) in THF (15 ml) was added Pd/C (245 mg, 20 mol %) and IPA (15ml), followed by the addition of a 37% aqueous solution of formaldehyde (0.93 ml, 11.50 mmol). The flask was then placed under vacuum before purging with hydrogen. The reaction was stirred at room temperature under a positive pressure of hydrogen for 5 hrs. The solution was filtered through a pad of celite, and washed with ethyl acetate. The solvent was removed *in vacuo* and column chromatography carried out using 3:7 EtOAc/Hexane, 5:5 EtOAc/Hexane, 7:3 EtOAc/Hexane.

Yield: 40-92% (yield is variable due to hydrolysis on silica gel during purification)
Found: $C_{16}H_{25}NO_9Na$ $[M+Na]^+$ *m/z* 398.1427 requires 398.1424
^1H NMR δ_H $CDCl_3$: 2.00 (3H, s, OAc), 2.02 (3H, s, OAc), 2.04 (3H, s, OAc), 2.07 (3H, s, OAc), 2.40 (6H, s, $N(CH_3)_2$), 3.57-3.62 (1H, m, 5-H), 4.03-4.07 (1H, d, 1-H, J = 9.3 Hz), 4.10-4.15 (1H, dd, 6-H', J = 12.1, 2.6 Hz), 4.20-4.26 (1H, dd, 6-H, J = 12.1, 4.9 Hz), 4.97-5.04 (1H, t, 4-H, J = 9.5 Hz), 5.08-5.15 (1H, t, 2-H, J = 9.4 Hz), 5.17-5.23 (1H, t, 3-H, J = 9.3 Hz).
^{13}C NMR δ_C $CDCl_3$: 20.9, 21.1, 21.2, 39.9, 62.9, 68.7, 69.4, 73.3, 74.5, 93.7, 169.9, 169.9, 170.6, 175.9, 178.4. *m/z* (ES +ve ion mode) 398 $[M+Na]^+$ 100 %.

General quaternisation reaction of dimethylamino glucose (4.94)

To a solution of **(4.94)** (49 mg, 0.13 mmol) in acetonitrile (1 ml) under argon was added the alkylhalide (0.08 ml, 1.33 mmol). The reaction was heated to 50^0C for 5hrs. The acetonitrile was removed *in vacuo* and a re-crystallisation carried out using ethanol.

2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl(trimethylammonium) iodide (4.96)



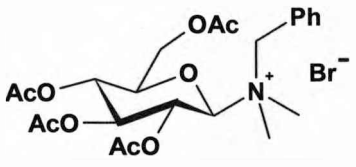
Yield: 51%

Found: C, 39.2; H, 5.5; N, 2.6 % $[M+H]^+$ *m/z* 390.1764 $C_{17}H_{28}NO_9$ requires C, 39.5; H, 5.4; N, 2.7 %; $C_{17}H_{29}NO_9$ requires *m/z* 390.1765

^{1}H NMR δ_H D_2O: 2.20 (3H, s, OAc), 2.22 (3H, s, OAc), 2.23 (3H, s, OAc), 2.28 (3H, s, OAc), 3.32 (9H, s, $N^+(CH_3)_3$), 4.38-4.42 (1H, m, 5-H), 4.48-4.52 (2H, m, 6-H', 6-H), 5.28-5.30 (1H, d, 1-H, J = 8.8 Hz), 5.34-5.38 (1H, t, 4-H, J = 9.2 Hz), 5.56-5.60 (1H, t, 3-H, J = 8.4 Hz), 5.73-5.77 (1H, t, 2-H, 8.6 Hz)

^{13}C NMR δ_C D_2O: 20.5, 20.6, 20.7, 20.8, 51.7, 62.1, 67.5, 68.4, 74.2, 75.2, 93.7, 172.2, 173.0, 173.2, 174.2. *m/z* (ES +ve ion mode) 390 $[M+H]^+$ 100 %.

2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl(benzyldimethylammonium) bromide (4.97)



The recrystallisation was carried out using ethyl acetate/hexane.

Yield: 64%

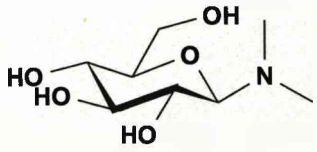
Mp: 160-162^{0}C. $C_{23}H_{32}NO_9$ Found $[M+H]^+$ *m/z* 466.2077 requires 466.2072.

^{1}H NMR δ_H D_2O: 2.17 (3H, s, OAc), 2.22 (6H, s, 2 x OAc), 2.28 (3H, s, OAc), 3.20 (3H, s, NCH_3), 3.32 (3H, s, NCH_3), 4.43 (1H, m, 5-H), 4.56 (2H, d, NCH_2), 4.64-4.71 (1H, dd, 6-H), 4.89-4.96 (1H, dd, 6-H'), 5.08-5.12 (1H, d, 1-H, J = 8.7 Hz), 5.32-5.40 (1H, t, 4-H, 9.3 Hz), 5.46-5.51 (1H, t, 3-H, J = 8.4 Hz), 5.73-5.82 (1H, t, 2-H, J = 8.4 Hz), 7.66-7.74 (5H, m, ArH).

^{13}C NMR δ_C D_2O: 20.5, 20.6, 20.7, 21.1, 47.6, 49.1, 62.4, 67.5, 68.0, 68.8, 74.2, 74.9, 91.1, 126.6, 130.1, 131.9, 133.4, 172.2, 173.0, 173.2, 174.2. *m/z* (ES +ve ion mode) 466 $[M+H]^+$ 100 %.

Deprotection of the dimethylamino sugar (4.94)

β-D-glucopyranosyl-N,N-dimethylamine- (4.108)



A solution of **(4.94)** (187 mg, 0.5 mmol) in MeOH (3 ml) was evacuated and flushed with nitrogen. NaOMe (5.4 mg, 0.1 mmol) was added to the reaction in MeOH (1 ml). The reaction was then left stirring at room temperature for 2hrs. Amberlite IR-120H ion exchange resin was then added to quench the reaction, and filtered off. The methanol was removed *in vacuo* to yield a clear, viscous oil.

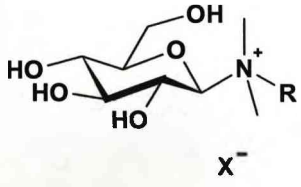
Yield: 100 %

Found [M+H] *m/z* 208.11803 for $C_8H_{18}O_5N$

^{1}H NMR δ_H d_6-DMSO: 2.31-2.33 (6H, s, N(CH_3)$_2$), 2.96-3.04 (2H, m, 5-H, 3-H), 3.10-3.17 (2H, m, 4-H, 2-H), 3.40-3.46 (1H, m, 6-H'') 3.62-3.68 (2H, m, 6-H', 1-H).

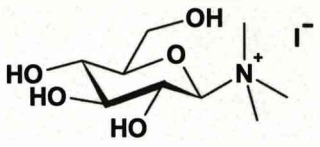
^{13}C NMR δ_C d_6-DMSO: 48.9, 61.8, 70.5, 70.9, 78.1, 78.5, 94.7 (anomeric)

General deprotection procedure for the quaternary ammonium salts



To a solution of quaternary ammonium salt (0.06 mmol) in MeOH (1 ml) under argon was added solid Na_2CO_3 (18 mg, 0.17 mmol) at room temperature, and was left stirring at room temperature for 3 hrs. The reaction was quenched with Amberlite H^+ resin (57 mg, 0.17 mmol) until the reaction was ~pH 7. The resin was filtered off and washed with methanol. The solvent was removed *in vacuo* and a recrystallisation carried out using ethanol and a few drops of ether on cooling.

β-*D*-Glucopyranosyl(trimethylammonium) iodide (4.99)

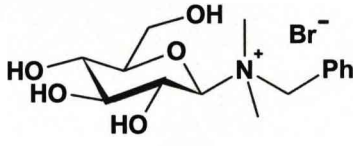


Yield: 87%, white crystalline solid

Found: C, 30.88, H, 5.78, N, 4.00 $[M]^+$ *m/z*, 222.1349. $C_9H_{20}NO_5I$ requires C, 30.94, H, 5.78, N, 4.01 %, $C_9H_{20}NO_5$ requires *m/z*, 222.1341; ^1H NMR: δ_H d_6-DMSO: 3.11 (9H, s, $(CH_3)_3N^+$), 3.10-3.40 (3H, m, 2-H, 3-H, 4-H), 3.48 (2H, m, 6-H, 6-H'), 3.71 (1H, m, 5-H) and 4.54 (1H, d, J = 8.9 Hz, 1-H).

^{13}C NMR: δ_C d_6-DMSO: 50.7, 60.8, 69.2, 70.5, 77.3, 80.6 and 95.4; *m/z* (ES +ve mode) 222 cation $[M]^+$, 100%.

β-*D*-Glucopyranosyl(benzyldimethylammonium) bromide (4.100)



Once the methanol was removed *in vacuo*, the residue was triturated with ethyl acetate to leave an amorphous glass.

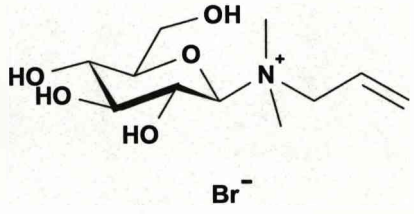
Yield: 72 %

Found $[M]^+$ *m/z* 298.1654 $C_{15}H_{24}NO_5$ requires 298.1660

^1H NMR δ_H d_6-DMSO: 3.09 (3H, s, $NC\underline{H}_3$) 3.12 (3H, s, $NC\underline{H}_3$), 3.13-3.19 (1H, t, 4-H, J = 9.3 Hz), 3.25-3.31 (1H, t, 3-H, J = 8.5 Hz), 3.36-3.42 (1H, m, 5-H), 3.52-3.59 (1H, dd, 6-H', J = 12.3, 6.6 Hz), 3.62-3.69 (1H, t, 2-H, J = 8.7 Hz), 3.81-3.87 (1H, dd, 6-H, J = 12.3, 1.7 Hz), 4.21-4.26 (1H, d, 1-H, J = 8.9 Hz), 4.54-4.60 (1H, d, $C\underline{H}_2Ph$, J = 12.5 Hz), 4.84-4.91 (1H, d, $C\underline{H}_2Ph$, J = 12.5 Hz), 7.49-7.57 (3H, m, ArH), 7.62-7.66 (2H, m, ArH)

^{13}C NMR δ_C d_6-Acetone: 46.9, 48.2, 61.1, 66.1, 69.2, 70.1, 77.2, 80.5, 92.9, 127.9, 129.4, 130.8, 133.4. *m/z* (ES + ion mode) $[M]^+$ 298 100 %.

β-*D*-Glucopyranosyl(dimethyl-2-propenyl)ammonium bromide (4.101)

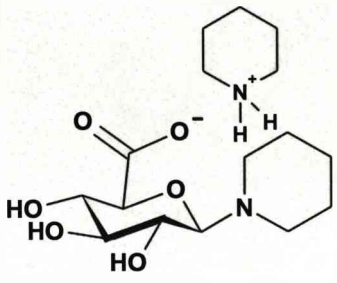


Yield: 98 %. (the starting material **(4.98)** was made by Chandrakala Pidathala)
Found: C, 38.1; H, 6.9; N, 3.4 %; $[M]^+$ *m/z*, 248.1492. $C_{11}H_{22}NO_5.H_2O$ requires C, 38.15; H, 6.9; N, 4.0 %; $C_{11}H_{22}NO_5$ requires *m/z*, 248.1498.
^1H NMR: δ_H $[(CD_3)_2SO]$: 2.97, 3.02 (6 H, 2s, 2xCH_3N^+), 3.16 (1 H, t, J = 9.4 Hz, 4-H), 3.32 (1 H, t, J = 8.7 Hz, 3-H), 3.30-3.40 (1 H, m, 5-H), 3.49 (1 H, dd, J = 12.4 and 6.1 Hz, 6-H), 3.56 (1 H, t, J = 8.8 Hz, 2-H), 3.72 (1 H, dd, J = 12.4 and 1.9 Hz, 6'-H), 3.90 (1 H, dd, J = 12.9, 7.2 Hz, one of $CHCH_2N^+$), 4.16 (1 H, dd, J = 12.8, 7.7 Hz, one of $CHCH_2N^+$), 4.39 (1 H, d, J = 8.9 Hz, 1-H), 5.62 (1 H, dd, J = 10.0 and 1.5 Hz, $CH=CH_2$ *cis*- olefinic H), 5.66 (1 H, dd, J = 17.2 and 1.5 Hz, $CH=CH_2$ *trans*- olefinic H), and 5.95 (1 H, m, $CH_2CH=CH_2$).
^{13}C NMR δ_C $[(CD_3)_2SO]$: 47.1, 48.3, 60.5, 65.9, 68.9, 70.1, 76.9, 80.1, 93.3, 125.4 and 129.0; *m/z* (ES +ve mode) 248 cation $[M]^+$ 100 %.

The reaction of free sugars and secondary amines

- Glucuronic acid derivatives

Piperidinium carboxylate-N-(piperidin-1-yl)-β-D-glucopyranuronate (4.128)



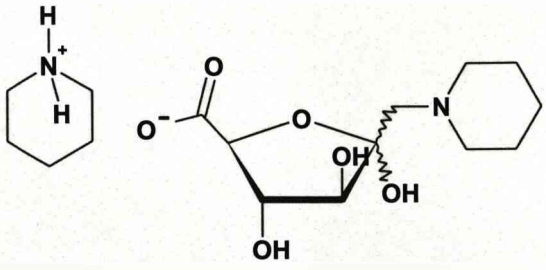
Glucuronic acid (1.94 g, 10 mmol) and piperidine (3.95 ml, 40 mmol) were heated together at 60^{0}C for 1hr with magnetic and manual stirring with a spatula. Acetone (25 ml) was added to the paste while the mixture was still warm and the acetone solution then filtered to remove any insoluble solid. The acetone solution was then allowed to cool to room temperature before storing the solution in the refrigerator (4^{0}C) overnight. During this time a white solid had precipitated which was collected.

Yield: 87%. 50:50 β and re-arranged product

Found $[M+H]^+$ *m/z* 262.128 for $C_{11}H_{20}O_6N$, C 54.91, N 8.86, 8.07 %, $C_{16}H_{30}N_2O_6$ requires C 55.49, H 8.67 N 8.09 %, $C_{11}H_{20}O_6N$ requires *m/z* 262.128.

^{1}H NMR δ_H d_6-DMSO β-anomer: 1.32-1.51 (6H, m, $NCH_2(C\underline{H}_2)_3$ of the aglycone), 1.56-1.67 (6H, m, $H_2N^+CH_2(C\underline{H}_2)_3$ of the salt), 2.50 (under DMSO) (2H, m, $C\underline{H}_2NC\underline{H}_2$ of the aglycone), 2.63-2.71 (1H, m, $C\underline{H}_2NCH_2$ of the aglycone), 2.76-2.84 (1H, m, CH_2-$NC\underline{H}_2$ of the aglycone), 2.95-3.01 (4H, m, $H_2N^+(C\underline{H}_2)_2$ of the salt), 3.05-3.12 (1H, t, 5-H, J = 9.29 Hz), 3.10-3.16 (1H, t, 4-H, J = 8.3 Hz), 3.18-3.27 (2H, m, 3-H, 2-H), 3.60-3.64 (1H, d, 1-H J = 8.8 Hz).

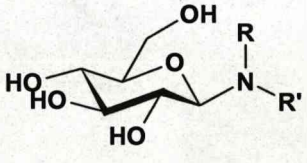
piperidinium(2S,3S,4S)-3,4,5-trihydroxy-5-(piperidin-1-ylmethyl)tetrahydrofuran-2-carboxylate



^{1}H NMR δ_H d_6-DMSO α and β re-arranged product: 1.32-1.51 (6H, m, $NCH_2(C\underline{H}_2)_3$ of the aglycone), 1.56-1.67 (6H, m, $H_2N^+CH_2(C\underline{H}_2)_3$ of the salt), 2.50 (under DMSO) (2H, m, $C\underline{H}_2NC\underline{H}_2$ of the aglycone), 2.63-2.71 (1H, m, $CH_2NC\underline{H}_2$ of the aglycone), 2.76-2.84 (1H, m, $C\underline{H}_2NCH_2$ of the aglycone), 2.95-3.01 (4H, m, $H_2N^+(CH_2)_2$ of the salt), 3.26-3.31 (0.5H, d, $C\underline{H}_2N$, J = 19 Hz), 3.39-3.43 (0.5H, d, $C\underline{H}_2N$, J = 19 Hz) 3.65-3.69 (1H, dd, 4.8, 1.8 Hz), 3.51-3.53 (0.5H, d, $C\underline{H}_2N$, J = 7.6 Hz), 3.58-3.60 (0.5H, d, $C\underline{H}_2N$), 3.66-3.67 (0.5H, dd, 2-H, J = 4.8, 1.6 Hz) 3.69-3.71 (1H, dd, 2-H, 4.0, 1.6 Hz), 3.8 (0.5H, d, 4-H, 1.3 Hz), 3.93-3.96 (0.5H, dd, 3-H, 4.8, 1.2 Hz), 4.02-4.03 (0.5H, d, 3-H, J = 4.0 Hz), 4.11-4.12 (0.5H, d, 4-H, J = 1.3 Hz).

^{13}C NMR δ_C d_6-DMSO re-arranged product and β-product: 22.4, 22.9, 24.7, 24.9, 25.8, 26.2, 26.2, 44.1, 48.6, 49.0, 54.4, 69.5, 70.8, 72.8, 76.6, 77.4, 78.1, 79.1, 81.2, 95.4, 102.1, 174.1, 174.1

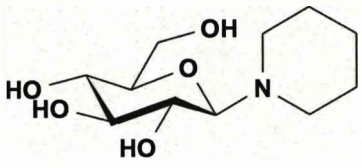
-Glucose derivatives



Glucose (1.8 g, 10 mmol) and secondary amine (20 mmol) were heated together (60^{0}C for morpholine, piperidine and N-methylpiperazine, 40^{0}C for pyrrolidine) for 1hr with magnetic and manual stirring with a spatula. Acetone (25ml) and methanol (2.5ml) were added to the paste while the mixture was still warm and the acetone solution then filtered to remove any insoluble solid. The acetone solution

was then allowed to cool to room temperature before storing the solution in the refridgerator (4^0C) overnight. Iso-hexane was added to the solution if no precipitation had occurred, until the solution was cloudy throughout.[20]

β-*D*-(glucopyranosyl) piperidine(4.111)[20]



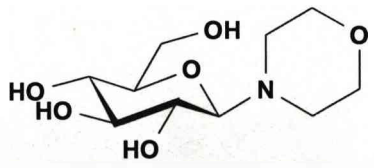
Yield: 89%

Mp: 123-125°C. Found: C, 53.30, H, 8.58, N, 5.65 %, $[M+H]^+$ *m/z* 248.149. $C_{11}H_{21}O_5N$ requires C, 53.43, H, 8.55, N, 5.66 % *m/z* $[M+H]^+$ 248.148

^{1}H NMR δ_H d_6-DMSO: 1.34-1.42 (2H, m, aglycone $CH_2C\underline{H}_2CH_2$), 1.44-1.52 (4H, m, $C\underline{H}_2CH_2C\underline{H}_2$), 2.46-2.55 under DMSO peak (2H, m, $C\underline{H}_2NC\underline{H}_2$), 2.78-2.86 (2H, m, $C\underline{H}_2NC\underline{H}_2$), 2.93-3.03 (2H, m, 4-H, 6-H), 3.09-3.16 (1H, t, 3-H, J = 7.8 Hz), 3.18-3.25 (1H, t, 2-H, J = 8.8 Hz), 3.36-3.44 (1H, m, 5-H), 3.60-3.68 (1H, d, 1H, J = 8.8 Hz, m, 6-H')

^{13}C NMR δ_C d_6-DMSO: 24.91, 26.22, 48.47, 61.82, 69.54, 70.90, 78.26, 78.76, 95.51 (anomeric). *m/z* (ES +ve ion mode) 248 $[M+H]^+$ 100 %.

β-*D*-(glucopyranosyl) morpholine (4.112)



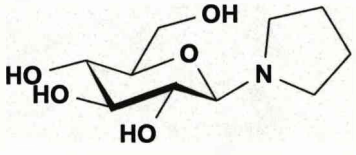
Yield: 79%

Found: $[M+H]^+$ *m/z* 250.128 $C_{10}H_{19}O_6N$ requires *m/z* 250.129

^{1}H NMR δ_H d_6-DMSO: 2.52-2.58 (2H, m, $C\underline{H}_2NC\underline{H}_2$), 2.79-2.86 (2H, m, $C\underline{H}_2NC\underline{H}_2$), 2.95-3.04 (2H, m, 4-H, 5-H), 3.12-3.17 (1H, t, 3-H, J = 8.5 Hz), 3.18-3.24 (1H, t, 2-H, 8.5 Hz) 3.39-3.45 (1H, dd, 6-H, J = 11.8, 5.5 Hz), 3.52-3.59 (4H, t, $C\underline{H}_2OC\underline{H}_2$, J = 4.8 Hz)) 3.62-3.68 (2H, m, 6-H', 1-H, J = 8.8Hz)

^{13}C NMR δ_C d_6-DMSO: 48.95 (morpholine), 61.6 (C6), 61.8 (morpholine), 67.7 (C4), 69.2 (C3), 70.8 (C2), 78.1 (C5), 94.6 (C1 anomeric). *m/z* (ES +ve ion mode) 250 $[M+H]^+$ 100 %.

β-*D*-(glucopyranosyl) pyrrolidine(4.114)



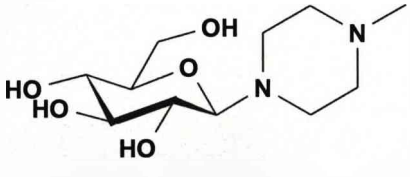
Yield: 86%

Found $[M+H]^+$ *m/z* 234.133 $C_{10}H_{20}O_5N$ requires *m/z* 234.133

^{1}H NMR δ_H d_6-DMSO: 1.65-1.70 (4H, m, CH_2CH_2), 2.70-2.77 (2H, m, CH_2NCH_2), 2.85-2.92 (2H, m CH_2NCH_2), 2.97-3.05 (2H, m, 3-H, 5-H), 3.09-3.14 (2H, m, 2-H, 4-H), 3.38-3.44 (1H, dd, 6-H, J = 11.8, 4.5 Hz), 3.63-3.69 (1H, d, 6-H', J = 11 Hz), 3.86-3.90 (1H, d, 1-H, J=8.5Hz)

^{13}C NMR δ_C d_6-DMSO: 24.2 (pyrroidine), 46.0 (pyrrolidine), 61.8 (C6), 71.0 (C3), 72.0 (C2), 78.1 (C4), 78.2 (C5), 90.9 (C1). *m/z* (ES +ve ion mode) 234 $[M+H]^+$ 100 %.

β-*D*-(glucopyranosyl) *N*-1-methyl piperazine (4.113)



Yield: 100%

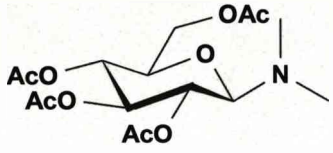
Found: C 48.03, H, 8.25, N, 9.79 % $[M+H]^+$ *m/z* 263.1607. $C_{11}H_{22}O_5N_2.H_2O$ requires C, 47.13, H, 8.62, N, 9.99 %, $C_{11}H_{23}O_5N_2$ requires *m/z* 263.1610

^{1}H NMR δ_H D_2O: 2.31 (NCH_3), 2.78-2.81 (4H, m, $MeN(CH_2)_2$), 3.01-3.10 (4H, m, $MeN(CH_2)_2$), 3.38-3.43 (2H, m, 4-H, 5-H), 3.5-3.58 (1-H, m, 3-H), 3.58-3.67 (1H, t, 2-H), 3.75-3.81 (1H, m, 6-H), 3.91-3.99 (1H, m, 6-H), 4.00-4.07 (1H, d, 1-H, J = 8.9 Hz)

^{13}C NMR δ_C D_2O: 44.5, 44.9, 54.5, 61.5, 69.4, 70.5, 77.7, 77.8, 93.8. *m/z* (ES +ve ion mode) 263 $[M+H]^+$ 100 %.

-Alterantive route for the synthesis of the acetylated dimethylamino glucose

2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl-N, N-dimethylamine (4.94)

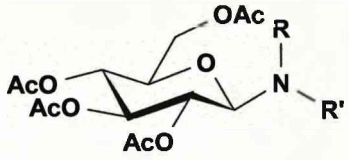


Glucose (1.8 g, 10 mmol) and 40 % w/v aqueous dimethylamine (20 mmol) were heated together at 40^0C for 1hr. The water and excess dimethylamine was removed *in vacuo* and NMR showed a mixture of product and glucose (2:1). The material was then dissolved in Pyridine (15 ml) and cooled to 0°C. Acetic anhydride (4.16 ml, 44 mmol) was then added and the reaction left at 0°C for 4 hrs. The reaction was then neutralised with $NaHCO_3$ (~ 20 ml) and diluted with DCM (50 ml). The aqueous phase was extracted twice more with DCM (50 ml) and the combined DCM layers washed with water (100 ml) and brine (100 ml). The DCM was then dried over Na_2SO_4 and the solvent removed *in vacuo*. Column chromatography was carried out using 40-70 % EtOAc/Hexane.

Yield: 23 %

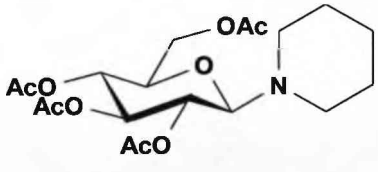
Data as before when using the reductive amination method.

- Acetylation of the cyclic amino sugars



To a solution of aminosugar (**(4.111)/(4.112)/(4.113)**) (2.77 mmol) in pyridine (2 ml) at 0°C was slowly added acetic anhydride (1.93 ml, 20.4 mmol). On full addition of the acetic anhydride the reaction was allowed to reach room temperature and was left stirring at for 4-5hrs. The pyridine was then removed under vacuum. DCM (20 ml) was added to the residue and the reaction quenched over ice with saturated $NaHCO_3$ (~20 ml) and water (~20 ml) until pH 7-8 was obtained. The DCM layer was collected and the aqueous phase extracted with DCM (3 x 20 ml). The DCM fractions were combined and washed with water (1 x 50 ml). The DCM was then dried over $MgSO_4$ and then removed *in vacuo*. A recrystallisation was carried out using hot ethanol, on cooling ether was added which brought on the formation of white crystals.

2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl piperidine (4.115)[20]



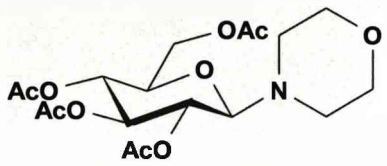
Found: C, 55.1, H, 7.12, N, 3.24 %, $[M+Na]^+$ *m/z* 438.171. $C_{19}H_{29}O_9N$ requires C, 54.94, H, 7.04, N, 3.37 %, $C_{19}H_{29}O_9NNa$ requires *m/z* 438.174

^1H NMR δ_H $CDCl_3$: 1.38-1.56 (6H, m, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$) 2.0-2.08 (12H, 4 x s, $OCOC\underline{H}_3$), 2.5-2.53 (2H, m, $C\underline{H}_2NC\underline{H}_2$), 2.88-2.93 (2H, m, $C\underline{H}_2NC\underline{H}_2$), 3.56-3.6 (1H, m ,5-H), 3.96-3.98 (1H, d, 1-H, J = 8.9 Hz), 4.09-4.12 (1H, dd, 6-H, J = 12.1, 2.6 Hz), 4.21-4.25 (1H, dd, 6-

H', J = 12.1, 4.8 Hz), 4.96-5.01 (1H, t, 4-H, J = 9.2 Hz), 5.13-5.17 (1H, t, 2-H, J = 9.4 Hz), 5.18-5.23 (1H, t, 3-H, J = 9.4 Hz)

^{13}C NMR δ_C $CDCl_3$: 21.0, 21.1, 21.2, 24.9, 26.7, 49.4, 62.8, 67.9, 69.4, 73.4, 74.3, 94.8, 169.9, 170.0, 170.7, 171.1

2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl morpholine (4.116)



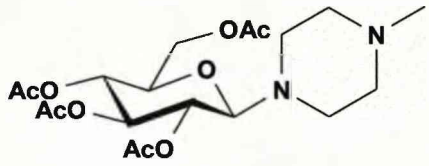
Yield: 68%

Found: C, 51.53, H, 6.44, N, 3.33 %, $[M+Na]^+$ *m/z* 440.1523 $C_{18}H_{27}O_{10}N$ requires C, 51.80, H, 6.47, N, 3.36 %, $C_{18}H_{27}O_{10}NNa$ requires *m/z* 440.1523

^{1}H NMR δ_H $CDCl_3$: 2.02 (3H, s, OAc), 2.03 (3H, s, OAc), 2.08 (3H, s, OAc), 2.11 (3H, s, OAc), 2.58-2.63 (2H, m, $C\underline{H}_2NC\underline{H}_2$), 2.93-3.01 (2H, m $C\underline{H}_2NC\underline{H}_2$), 3.53-3.66 (5H, m, $C\underline{H}_2OC\underline{H}_2$ 5-H), 3.96-4.01 (1H, d, 1-H, J = 9.3 Hz), 4.95-5.02 (1H, t, 4-H, J = 9.6 Hz), 5.10-5.17 (1H, t, 2-H, J = 9.4 Hz), 5.21-5.28 (1H, t, 3-H, J = 9.4 Hz).

^{13}C NMR δ_C $CDCl_3$: 20.7, 20.8, 20.8, 20.9, 47.0, 61.8, 67.5, 69.3, 73.0, 73.1, 73.4, 93.8, 169.9, 170.3, 170.4, 170.8. *m/z* (ES +ve ion mode) 440 $[M+Na]^+$ 100 %.

2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl N-1-methyl piperazine



Yield: 26%

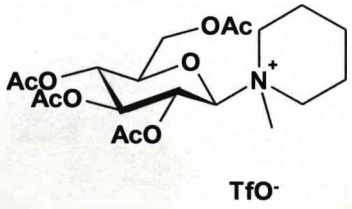
Found: $[M+H]^+$ *m/z* 431.2030. $C_{19}H_{31}O_9N_2$ requires *m/z* 431.2017.

^{1}H NMR δ_H $CDCl_3$: 2.02 (3H, s, OAc), 2.03 (3H, s, OAc), 2.08 (3H, s, OAc), 2.11 (3H, s, OAc), 2.26 (3H, s, NCH_3), 2.29-2.43 (4H, m, $MeN(CH_2)_2$), 2.09-2.18 (2H, m, CH_2NCH_2), 2.97-3.05 (2H, m, CH_2NCH_2), 3.61-3.68 (1H, m, 5-H), 3.99-4.03 (1H, d, 1-H, J = 9.3 Hz), 4.08-4.14 (1H, dd, 6-H', J = 12.1, 2.6 Hz), 4.19-4.22 (1H, dd, 6-H, J = 12.1, 5.2 Hz), 4.95-5.00 (1H, t, 4-H, J = 9.5 Hz), 5.09-5.13 (1H, t, 2-H, J = 9.5 Hz), 5.20-5.25 (1H, t, 3-H, J = 9.4 Hz)

^{13}C NMR δ_C $CDCl_3$: 20.7, 20.8, 20.9, 20.9, 21.0, 46.2, 55.6, 62.7, 68.3, 68.7, 73.3, 74.1, 93.6, 169.9, 170.4, 170.5, 170.9. *m/z* (ES +ve ion mode) 431 $[M+H]^+$ 100 %.

-Quaternisation of the piperidine derivative

(N-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-N-methyl)piperidinium trifluoromethanesulfonate (4.116)



To a solution of **(4.115)** (100 mg, 0.24 mmol) in DCM (2 ml) under nitrogen was added MeOTf (0.054 ml, 0.48 mmol). The reaction was then left stirring for 4 hrs at room temperature. The DCM was removed *in vacuo*. Column chromatography was carried out using 100 % EtOAc, 50:50 Ethanol/DCM. The ethanol/DCM fractions were combined and solvent removed to give the desired product.

N.B. The product is undetectable by anisaldehyde stain when diluted i.e. product can not been seen when diluted in the eluting solvent of the column, evaporation of the solvent showed product present.

Yield: 62 %

Found: *m/z*, 430.2064. $C_{20}H_{32}NO_9$ requires *m/z*, 430.2077;

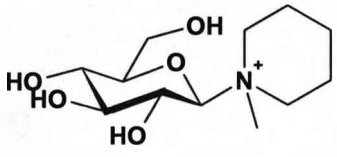
^{1}H NMR δ_H $CDCl_3$: 1.70-1.80 (3H, m, CCH_2C), 1.98, 2.03, 2.05 and 2.12 (12 H, 4s, 4xCH_3CO), 2.00-2.10 (3 H, m, CCH_2C), 3.30 (3 H, s, CH_3N^+), 3.60-3.95 (4 H, 2m, 2xCH_2N^+), 4.27 (1 H, dd, 6-H, J = 12.6, 5.9 Hz), 4.36-4.44 (2 H, m, 6-H', 5-H), 5.23 (1 H, t, 4-H, J = 9.7 Hz), 5.49 (1 H, dd, 3-H, J = 9.4, 8.3 Hz), 5.69-5.78 (2 H, m, 1-H, 2-H).

^{13}C NMR: δ_C $CDCl_3$: 20.7, 20.9, 21.0 (x 2), 21.3, 21.8, 61.8, 62.2, 62.6, 68.4, 68.9, 74.6, 76.5, 90.9; 117.7, 120.9, 124.1, 127.3 (CF_3, q), 170.5 (x 2), 170.6 and 171.3;

m/z (ES +ve ion mode) 430 cation $[M]^+$ 100 %.

-Deprotection of the quaternary ammonium salt

(N-(β-D-glucopyranosyl)-N-methyl)piperidinium trifluoromethanesulfonate(4.117)



TfO^-

To a solution of **(4.116)** (394 mg, 0.92 mmol) in MeOH (6 ml) was added NaOMe (2.3 mg, 0.046 mmol). The reaction was left stirring at room temperature for 5 hrs. The reaction was then quenched with Amberlite H^+ resin to reach pH 7. The resin was then filtered off and washed with methanol. The solvent was then removed *in vacuo*.

Yield: 100 %

Found: *m/z* 262.1661. $C_{12}H_{24}NO_5$ requires *m/z*, 262.1654.

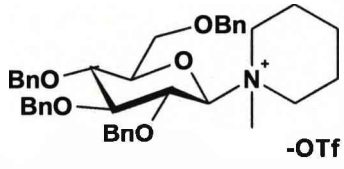
^{1}H NMR: δ_H d_6-Acetone: 1.69-1.78, 1.91-2.03, 2.05-2.12 (6 H, 3m, CCH_2C), 3.27 (3 H,

s, CH_3N^+), 3.48 (1 H, t, J = 9.5 Hz, 4-H), 3.45-3.75 (2 H, m, CH_2N^+), 3.65-3.75 (2 H, m, 2x6-H), 3.67 (1 H, t, J = 9.3 Hz, 3-H), 3.87-3.98 (2 H, m, CH_2N^+), 3.90 (1 H, t, J = 8.8 Hz, 2-H), 4.12 (1 H, m, 5-H) and 5.00 (1 H, d, J = 8.8 Hz, 1-H).

^{13}C NMR: δ_C d_6-Acetone: 20.7, 20.8, 22.0, 45.5, 61.1, 61.8, 61.9, 70.2, 71.6, 78.7, 81.7, 93.5, 118.3 (CF_3), 121.5 (CF_3) N.B. only two peaks from the CF_3 signal can be assigned due to weak spectrum. *m/z* (ES +ve mode) 262 cation $[M]^+$ 100 %.

-Formation of the benzyl protected piperidinium sugar

(N-(2,3,4,6-tetra-O-benzyl)-β-D-glucopyranosyl)-N-methyl)piperidinium trifluoromethanesulfonate (4.89)



To a solution of **(4.86)** (598 mg, 1.07 mmol) in DCM (5 ml) was added piperidine (0.21 ml, 2.14 mmol). The reaction was left stirring at RT for 17 hrs. The precipitate was then filtered off, and the solvent then removed *in vacuo*. The residue was then taken up in a solution of DCM (5 ml) and MeOTf (0.24 ml, 2.14 mmol) was added to the solution. The solution was left stirring at RT for 17 hrs. The reaction was then quenched with water (10 ml) followed by sat.$NaHCO_3$(aq) (2 ml) and diluted with DCM (20 ml). The organic layer was separated and the aqueous layer extracted with DCM (2 x 20 ml). The combined organic layers were then extracted with water (2 x 20 ml), dried over Na_2SO_4 and concentrated *in vacuo*. Column chromatography was then carried out using 10-20 % EtOH/DCM.

Yield: 38 %

Found: $[M]^+$ *m/z* 622.353. $C_{40}H_{48}O_5N$ requires *m/z* 622.353

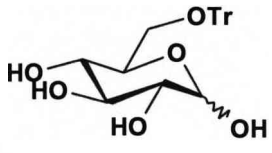
^1H NMR δ_H d_6-acetone: 1.62-1.77 (2H, m, $CH_2C\underline{H_2}CH_2$), 1.79-2.08 (4H, m, $C\underline{H_2}CH_2C\underline{H_2}$-), 3.25 (3H, s, $CO_2C\underline{H_2}$), 3.5-3.66 (2H, m, $C\underline{H_2}NC\underline{H_2}$), 3.81-3.92 (3H, m, $C\underline{H_2}NC\underline{H_2}$, 4-H),

4.08-4.12 (1H, m 5-H), 4.15-4.19 (1H, t, 3-H, J = 7.8 Hz), 4.31-4.36 (1H, t, 2-H, J = 7.3 Hz), 4.58-4.66 (2H, 2 x d, C$\underline{H}_2$OBn, J = 12.1 Hz), 4.68-4.72 (1H, d, C$\underline{H}_2$Bn, J = 11.3 Hz), 4.8-4.84 (1H, d, C$\underline{H}_2$Bn, J = 11.2 Hz), 4.86-5.01 (3H, 3 x d, C$\underline{H}_2$Bn, J = 11.3 Hz), 5.07-5.12 (1H, d, C$\underline{H}_2$Bn, J = 11.3 Hz), 5.28-5.32 (1H, d, 1-H, J = 8.2 Hz), 7.28-7.46 (20H, m , Ar$\underline{H}$). ES (+ve ion mode) $[M]^+$ 622 100 %

^{13}C NMR δ_C d_6-acetone: 20.8 (x 2), 21.9, 45.6, 61.3, 61.5, 69.9, 74.1, 74.5, 75.1, 75.8, 77.5, 78.0, 79.6, 85.2, 93.3, 117.9, 121.2, 124.4, 127.6 (q for CF_3), 128.8, 128.9, 129.0, 129.1, 129.2 (x 2), 129.6 (x 2), 129.8, 138.7, 139.5, 139.6, 139.8.

The 6-O-trityl glucose series

6-O-Trityl-α,β-D-glucopyranose (4.119)[21]



To a solution of Glucose (5 g, 27.78 mmol) in pyridine (50 ml) at 0°C was added Tritylchloride (8.5 g, 30.55 mmol) and the reaction left stirring at 0°C for 7 hours. The reaction was then diluted with ethyl acetate (100 ml) and water (100 ml). The water layer was then extracted with ethyl acetate (3 x 100 ml). The combined ethyl acetate layers were then washed with brine (100 ml) and water (100 ml), and then dried over Na_2SO_4. The solvent was then removed *in vacuo*. Column chromatography was carried out using 5-10% EtOH in DCM to give a white solid.

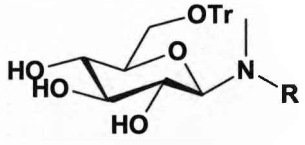
Yield 85%

Found: [M+Na] *m/z* 445.163. $C_{25}H_{26}O_6Na$ requires *m/z* 445.1627.

^{1}H NMR δ_H d_3-Acetonitrile ratio α/β 1:1: 3.06-3.57 (9H, m, α and β 3-H, 4-H, 6-H, 6'-H, 1 x 5-H), 3.84-3.89 (1H, m, 5-H), 4.44-4.47 (2H, m, α and β 2-H), 4.76-4.77 (1H, d, β1-H, J = 6.6 Hz), 5.10-5.12 (1H, dd, α1-H, J = 3.9 Hz), 7.22-7.27 (6H, m, α and β-ArH), 7.29-7.34 (12H, m, α and β-ArH), 7.46-7.50 (12H, m, α and β-ArH).

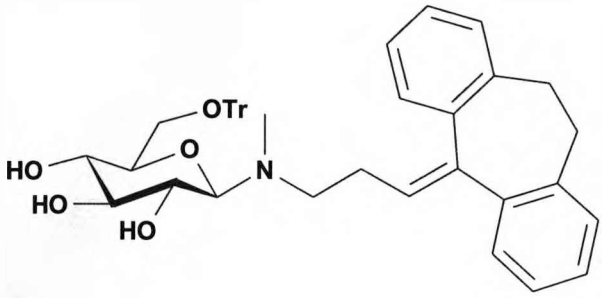
^{13}C NMR δ_C $CDCl_3$: 70.7, 71.6, 71.8, 72.4, 73.8, 74.8, 76.6, 87.2, 87.5, 92.7, 96.8, 127.4, 127.5, 128.2, 128.3, 129.1, 143.9, 144.1.

- General procedure for the condensation reaction



To a solution of 6-*O*-trityl glucose (1.2 g, 2.83 mmol) in DCM (15 ml) under nitrogen was added the secondary amine (2.83 mmol). The reactants were stirred at RT for 24 hrs. The DCM was then removed *in vacuo*. Column Chromatography was carried out using 100 % EtOAc.

(N-(6-O-Trityl)-β-D-glucopyranosyl))-N-methyl-3-(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-ylidene)-1-propanamine (4.122)



Yield 49%

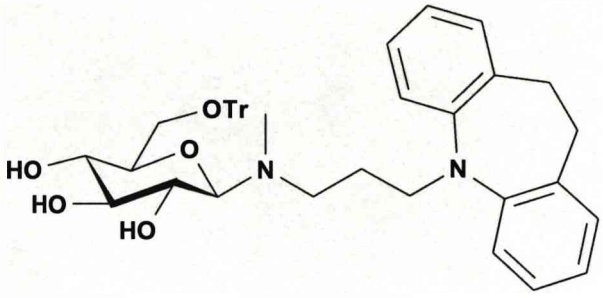
Found: Mp: 91-92°C. *m/z*, 668.3407. $C_{44}H_{46}NO_5$ requires *m/z*, 668.3376.

^1H NMR δ_H (CD_3CN + ~2 % D_2O) @343K: 2.40-2.47 (2 H, m, =CHC<u>H</u>$_2$), 2.40 (3 H, s, NCH_3), 2.92 (2 H, m, NC<u>H</u>$_2$$CH_2$), 2.95-3.15 (4 H, br m, ArC<u>H</u>$_2$C<u>H</u>$_2$Ar), 3.15-3.2 (1 H, dd, 6-H, J = 10.0 and 5.5 Hz), 3.25-3.32 (3 H, m, 4-H, 5-H, 6'-H), 3.31-3.35 (1 H, t, 3-H, J = 8.6 Hz), 3.38-3.42 (1 H, t, 2-H J = 8.5 Hz), 3.89-3.92 (1 H, d, 1-H, J = 8.8 Hz), 5.91-5.97 (1 H, t, J = 7.6 Hz, C=C<u>H</u>CH_2), 7.05-7.35 (17 H, m, ArH) and 7.49-7.54 (7 H, m, ArH).

^{13}C NMR δ_C (CD_3CN): 27.6, 31.2, 33.0, 34.3, 53.3, 63.4, 69.5, 70.1, 76.1, 77.4, 85.7,

93.6, 125.4, 125.5, 126.7, 126.8, 127.1, 127.4, 127.6, 127.7, 128.0, 128.3, 129.0, 129.7, 136.6, 139.1, 139.8, 140.8, 143.1, 144.0; *m/z* (ES +ve mode) 668 $[MH]^+$ 100 %.

(N-(6-O-Trityl)-β-D-glucopyranosyl))-N-methyl-3-(10,11-dihydro-5H-dibenzo[b,f]azepin-5-yl)-1-propanamine



Yield 29%

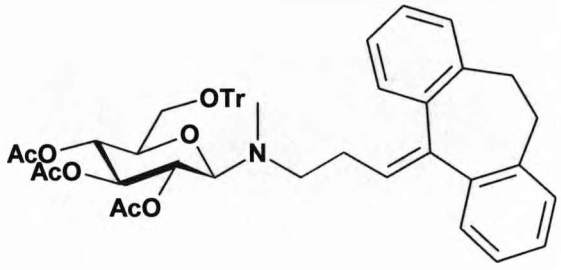
Found: [M+H] *m/z* 671.3503; $C_{43}H_{47}N_2O_5$ requires *m/z* 671.3485.

^{1}H NMR δ_H CD_3CN: 1.75-1.83 (2H, m, $CH_2C\underline{H}_2CH_2$, J = 6.9 Hz), 2.37 (3H, s, $NC\underline{H}_3$), 2.77-2.90 (2H, m, $NC\underline{H}_2CH_2$), 3.02-3.07 (1H, dd, 6-H, J = 9.9, 6.1 Hz), 3.07 (4H, s, $ArC\underline{H}_2C\underline{H}_2Ar$), 3.11-3.24 (4H, m, 6'-H, 4-H, 3-H, 5-H), 3.25-3.3 (1H,t, 2-H, J = 8.6 Hz) 3.76-3.82 (3H, t and d, $C\underline{H}_2N$, J = 6.7 Hz, 1-H, J = 8.8 Hz), 6.87-6.92 (2H, m, Ar$\underline{H}$), 7.06-7.13 (6H, m, Ar$\underline{H}$), 7.20-7.31 (9H, m, Ar$\underline{H}$), 7.46-7.50 (6H, m, Ar$\underline{H}$).

^{13}C NMR δ_C CD_3CN: 25.7, 31.5, 34.6, 47.5, 51.0, 63.5, 69.6, 70.2, 76.1, 77.6, 85.6, 93.8, 119.5, 122.1, 126.0, 126.6, 127.4, 128.3, 129.4, 133.8, 144.0, 148.1. *m/z* (ES +ve ion mode) $[M+H]^+$ 671 100 %.

-Acetylation of the free hydroxyl groups

(N-[(6-O-Trityl)-2,3,4-tri-O-acetyl)-β-D-glucopyranosyl))-N-methyl-3-(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-ylidene)-1-propanamine (4.123)



To a solution of **(4.122)** (1 g, 1.5 mmol) in pyridine (15 ml) at 0°C under nitrogen was added acetic anhydride (0.85 ml, 9 mmol). The reaction was allowed to reach room temperature on addition of the acetic anhydride and left stirring for 4 hrs. The reaction was then basified using sat. $NaHCO_3$ until pH 7 was reached. The reaction was then diluted with DCM (60 ml). The aqueous layer was then extracted with DCM (3 x 50 ml). The combined DCM layers were then washed with brine, (100 ml), water (100 ml) and dried over Na_2SO_4.

Yield: 79 %

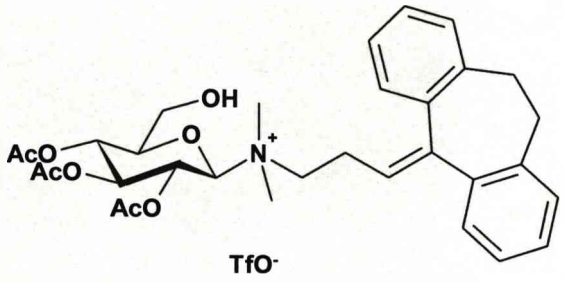
Found: *m/z*, 794.371. $C_{50}H_{52}NO_8$ requires *m/z*, 794.3693.

^{1}H NMR: δ_H (CD_3CN): 1.73, 1.77 and 1.95 (9 H, 3s, 3 x CH_3CO), 2.39 (5 H, m, =CHC$\underline{H}_2$ + NCH_3), 2.75-3.20 (6 H, m, ArC$\underline{H}_2$C$\underline{H}_2$Ar + NC$\underline{H}_2$C$\underline{H}_2$), 3.25-3.60 (3 H, m, 2 x 6-H, 5-H), 4.15-4.35 (1 H, br d, 1-H), 5.10-5.25 (3 H, br m, 2-H, 3-H, 4-H), 5.87 (1 H, t, =C$\underline{H}$CH$_2$), 7.00-7.35 (15 H, m, ArH), 7.47 (6 H, approx d, ArH); the ^{1}H NMR spectrum showed very broad signals and meaningful coupling constants cannot be quoted.

^{13}C NMR δ_C (CD_3CN): 19.4, 19.6, 22.0, 27.4, 31.0, 31.3, 33.0, 33.4, 61.6, 67.9, 68.1, 73.4, 73.6, 85.7, 92.2, 125.3, 125.7, 126.7, 127.1, 127.5, 127.7, 128.1, 128.2, 129.2, 129.7, 136.7, 139.1, 139.8, 141.0, 142.9, 143.6, 168.6, 169.0 and 169.6. *m/z* (ES +ve mode) 794 $[MH]^+$ 100 %.

-Quaternisation, and subsequent 6-O- trityl deprotection

(N-(2,3,4-tri-O-acetyl)-β-D-glucopyranosyl))-N, N-dimethyl-3-(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-ylidene)-1-propanammonium) trifluoromethanesulfonate (4.124)



To a solution of **(4.123)** (300 mg, 0.38 mmol) in DCM (8 ml) under nitrogen was added MeOTf (0.086 ml, 0.76 mmol). The reaction was left stirring at room temperature for 17 hrs. The DCM was removed *in vacuo* and column chromatography carried out using 100 % EtOAc, 20 % EtOH/DCM.

Yield: 59 %

Found: *m/z*, 566.2470. $C_{32}H_{40}NO_8$ requires *m/z*, 566.2754; ν_{max} (diamond) cm^{-1} 3347 (s, br), 1751, 1604, 1520 and 1452.

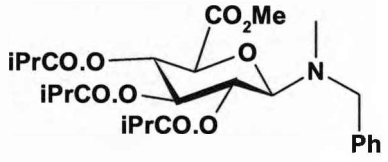
^{1}H NMR: δ (CD_3CN): 2.01, 2.03 and 2.09 (9 H, 3d, 3 x CH_3CO; fluxional effects), 2.64 (2 H, m, =CHC$\underline{H}_2$), 3.02, 3.04 (6 H, 2d, 2 x C$\underline{H}_3N^+$), 2.95-3.30 (5H, br m, ArC$\underline{H}_2$C$\underline{H}_2$Ar, NC$\underline{H}_2$), 3.30-3.45 (1 H, br m, 6-H), 3.50-3.70 (3 H, br m, 6-H', 5-H, NC$\underline{H}_2$), 4.86 (1 H, approx. d, 1-H, J = 6.5 Hz), 5.08 (1 H, approx. t, 4-H, J = 9 Hz), 5.26 (1 H, approx. t, 3-H, J = 9 Hz), 5.41 (1 H, dd, 2-H, J = 9.2 and 6.5 Hz), 5.79 (1 H, d, =C$\underline{H}CH_2$, J = 6.4 Hz) and 7.05-7.45 (8H, m, ArH).

^{13}C NMR: δ_C (CD_3CN): 19.5, 19.6, 19.8, 22.5, 31.2, 32.9, 48.3, 59.2, 63.6, 66.3, 67.0, 72.8, 77.5, 91.0, 118.9 (CF_3), 122.8 (CF_3), 125.9, 127.1, 127.4, 127.7, 128.1, 130.0, 136.9, 138.6, 139.1, 169.3, 169.6 and 169.8. N.B. only signals 118.9 and 122.8 could be assigned for the CF_3 quartet due to weak spectrum. *m/z* (ES +ve mode) 566 cation $[M]^+$, 100 %.

Using the hemiacetal in the glucuronic acid series

The condensation reaction of the hemiacetal with secondary amines

Methyl ((2,3,4-tri-O-isobutyryl)-β-D-1-N-methylbenzylamine)-glucopyranuronate



To a solution of hemiacetal **(4.129)** (300 mg, 0.72 mmol) in toluene (3 ml) under N_2 was added N-methylbenzylamine (0.18 ml, 1.43 mmol). The reaction was heated to 50°C for 5 hrs and the solvent then removed *in vacuo*. The residue was recrystallised from hexane/ether (9:1).

Yield: 29 %

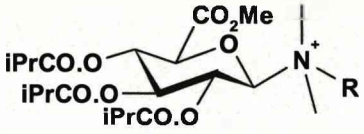
Found: C, 62.27, H, 7.61, N, 2.72, $[M+Na]^+$ *m/z* 544.2512. $C_{27}H_{39}O_9N$ requires C, 62.18, H, 7.53, N, 2.69; $C_{27}H_{39}O_9NNa$ requires *m/z* 544.2523.

^{1}H NMR δ_H $CDCl_3$: 1.06-1.21 (18H, m, $CH(C\underline{H}_3)_2$), 2.39 (3H, s, $NC\underline{H}_3$), 2.45-2.55 (3H, m, $C\underline{H}(CH_3)_2$), 3.74 (3H, s, $CO_2C\underline{H}_3$), 3.77-3.81 (1H, d, $C\underline{H}_2Ar$, J = 13.7 Hz), 3.87-3.94 (2H, 2 x d, $C\underline{H}_2Ar$, J = 13.7 Hz, 5-H, J = 9.9 Hz), 4.18-4.20 (1H, d, 1-H, J = 9.23 Hz), 5.17-5.20 (1H, t, 4-H, J = 9.8 Hz), 5.27-5.29 (2H, m, 2-H, 3-H), 7.21-7.31 (5H, m, ArH).

^{13}C NMR δ_C $CDCl_3$: 19.1, 19.2, 19.2, 19.3, 19.3, 34.2, 34.3, 34.3, 35.6, 53.0, 58.2, 67.8, 69.9, 73.2, 74.5, 92.9, 127.5, 128.6, 128.9, 138.9, 168.3, 175.7, 175.8, 176.3.

m/z (ES +ve ion mode) 544 $[M+Na]^+$ 100 %.

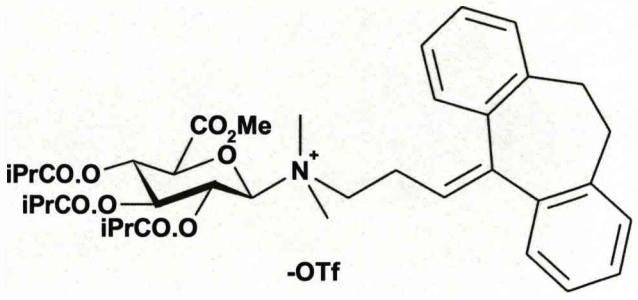
- Condensation reaction followed by acetylation and quaternisation using drug examples.



-OTf

To a solution of **(4.129)** (498 mg, 1.19 mmol) in toluene (5 ml) at 50°C was added Nortriptyline, Desipramine or the desmethyl AZ compound, **(4.136)** (1.19 mmol) in a solution of toluene (5 ml). The reaction was heated to 50°C under a nitrogen atmosphere for 24 hrs. The toluene was then removed *in vacuo* and the residue taken up in a solution of Pyridine (15 ml). The solution was cooled to 0°C and placed under nitrogen. Acetic anhydride (0.24 ml, 2.39 mmol) was then added slowly to the reaction mixture. The solution was then left stirring at 0°C for 4 hrs. The reaction was then quenched with $NaHCO_3$ until pH 7-8 was achieved. The solution was then diluted with DCM (20 ml) and the organic layer collected. The aqueous phase was then extracted with DCM (3 x 50 ml) and the organic layers combined. The combined organic layers were then washed with brine (50 ml) and water (2 x 50 ml) and then dried over Na_2SO_4. The organic solvent was then removed *in vacuo* with the pyridine residues being removed under high vacuum. The residue was then dissolved in dry DCM (8 ml) and evacuated with nitrogen. Methyl triflate (0.27 ml, 2.39 mmol) was then added to the stirred solution. On complete addition the reaction was left stirring for 17 hrs at RT. The DCM was then removed *in vacuo* and column chromatography carried out using, 100 % EtOAc, 10:90 IPA/DCM.

Methyl(N-(2,3,4-tri-O-isobutyryl)-β-D-glucopyranuronate))-N, N-dimethyl-3-(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-ylidene)-1-propanammonium trifluoromethanesulfonate (4.130)

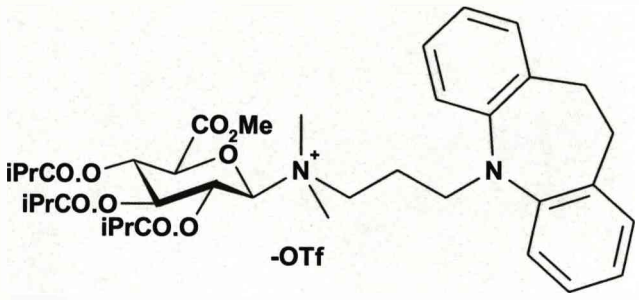


Yield: 18 %. Found: C 57.78, H 6.38, N 1.44 %, $[M]^+$ *m/z* 678.3660; $C_{40}H_{52}O_{12}NSF_3$ requires C 58.03, H 6.33, N 1.69 % $[C_{39}H_{52}O_9N]^+$ requires *m/z* 678.3642

^{1}H NMR δ_H d_6-DMSO: 1.00-1.06 (18H, m, $CO_2CH(C\underline{H}_3)_2$), 2.40-2.49 (3H, m, $CO_2C\underline{H}(CH_3)_2$), 2.50-2.58 (2H, broad s, $CHC\underline{H}_2CH_2N^+$), 2.73-2.92 (2H, broad s, $C\underline{H}_2C\underline{H}_2$), 2.98-3.09 (6H, 2 x d, $N^+(C\underline{H}_3)_2$), 3.2-3.31 (2H, broad s, $C\underline{H}_2C\underline{H}_2$), 3.34 (3H, s, $CO_2C\underline{H}_3$), 3.62-3.71 (2H, m, $CHCH_2C\underline{H}_2N^+$), 4.5-4.52 (1H, m, 5-H), 5.14-5.30 (1H, m, 3-H), 5.3-5.4 (1H, m, 4-H), 5.5-5.51 (1H, t, 2-H, J = 9.2 Hz), 5.7-5.84 (2H, t, $C\underline{H}CH_2CH_2N^+$ J = 7.2 Hz and, broad m, 1-H), 7.08-7.3 (8H, m, ArH).

^{13}C NMR δ_C $CDCl_3$: 14.3, 18.2, 18.6, 18.7, 18.8, 18.9, 22.4, 23.0, 31.6, 33.4, 33.5, 33.5, 33.6, 48.8, 48.9, 53.2, 67.0, 67.7, 72.2, 72.7, 126.3, 126.4, 127.6, 127.9, 128.3, 128.5, 128.6, 128.7, 130.5, 137.0, 139.0, 139.3, 140.4, 174.7, 175.1, 175.4 (x2). *m/z* (ES +ve ion mode) 678 $[M]^+$ 100 %.

Methyl(N-(2,3,4-tri-O-isobutyryl)-β-D-glucopyranuronate))-N, N-dimethyl-3-(10,11-dihydro-5H-dibenzo[b,f]azepin-5-yl)-1-propanammonium trifluoromethane sulfonate (4.131)

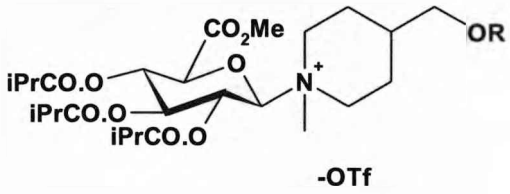


Yield: 16 %. Found: $[M]^+$ *m/z* 681.376, $C_{38}H_{53}O_9N_2$ requires *m/z* 681.375

^{1}H NMR δ_H $CDCl_3$: 1.05-1.08 (18H, m, $CO_2CH(C\underline{H}_3)_2$), 2.1-2.2 (2H, m, $NCH_2C\underline{H}_2CH_2$), 2.35-2.6 (3H, m, $CO_2C\underline{H}(CH_3)_2$), 2.95 (3H, s, $NC\underline{H}_3$), 3.08 (3H, s, $NC\underline{H}_3$), 3.18 (4H, s, $C\underline{H}_2C\underline{H}_2$), 3.4-3.5 (1H, m, $NC\underline{H}_2CH_2CH_2$), 3.55-3.65 (1H, m, $NC\underline{H}_2CH_2CH_2$), 3.76 (3H, s, $CO_2C\underline{H}_3$), 3.8-3.98 (2H, m, $NCH_2CH_2C\underline{H}_2$), 4.5-4.53 (1H, d, 5-H, J = 9.5 Hz), 5.09-5.18 (1H, t, 4-H, J = 9.3 Hz), 5.3-5.5 (2H, 2 x t, 2-H, J = 8.4 Hz, 3-H, J = 8.4 Hz), 5.6-5.65 (1H, d, 1-H, J = 8.6 Hz), 6.9-7.23 (8H, m, ArH).

^{13}C NMR δ_C $CDCl_3$: 18.5, 18.8, 18.9, 21.1, 32.4, 34.1, 34.2, 34.3, 46.7, 48.2, 48.9, 53.4, 60.9, 67.4 (4-C), 68.2 (3-C), 72.8 (2-C), 74.0 (5-C), 93.6 (1-C), 120.0, 123.9, 127.2, 130.6, 134.6, 147.2, 166.3, 175.0, 175.9 (x2). *m/z* (ES +ve ion mode) 681 $[M]^+$ 100 %.

AZ N⁺-Glucuronide (4.135)



Yield: 23 %

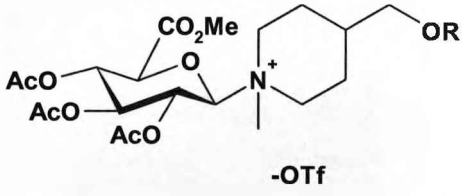
Found: $[M]^+$ *m/z* 877.294, $C_{41}H_{53}O_{11}N_4{}^{81}BrF$ requires *m/z* 877.285

^1H NMR δ_H d_6-acetone: 1.04-1.21 (18H, m, $CH(C\underline{H}_3)_2$), 1.23-1.42 (4H, m, $C\underline{H}_2CHC\underline{H}_2$), 1.76-1.9 (1H, m, $C\underline{H}CH_2OR$), 2.42-2.53 (3H, m, $C\underline{H}(CH_3)_2$), 2.87-2.93 (3H, m, $C\underline{H}_2NC\underline{H}_2$-), 3.21-3.3 (1H, m, $C\underline{H}_2NCH_2$), 3.68 (3H, s, $NC\underline{H}_3$), 3.72 (3H, s, $CO_2C\underline{H}_3$), 3.92 (3H, s, aglycone), 4.24-4.38 (1H, d, 5-H, J = 10 Hz), 4.43-4.46 (1H, d, 1-H, J = 9.4 Hz), 5.08-5.13 (1H, t, 4-H, J = 9.8 Hz), 5.17-5.24 (1H, t, 2-H, J = 9.7 Hz), 5.37-5.42 (1H, t, 3-H, J = 9.6 Hz), 7.0-7.8 (6H, aglycone).

^{13}C NMR δ_C d_6-acetone: 19.4, 19.6, 19.7, 34.9, 35.0, 35.1, 37.3, 37.7, 45.1, 53.1, 56.8, 68.2, 71.0, 73.7, 74.8, 75.0, 95.0, 100.9, 104.2, 108.5, 113.7, 114.1, 119.5, 127.6, 127.9, 135.4, 135.8, 149.8, 150.7, 154.5, 154.9, 155.7, 157.6, 169.1, 176.1, 176.2, 176.4.

-Using the acetate protected hemiacetal

Protected AZ N⁺-Glucuronide (4.137)



The method is as above replacing **(4.129)** for **(4.61).**

Yield: 14 %

Found: $[M]^+$ *m/z* 791.190 $C_{35}H_{41}O_{11}N_4{}^{79}BrF$ requires *m/z* 791.193

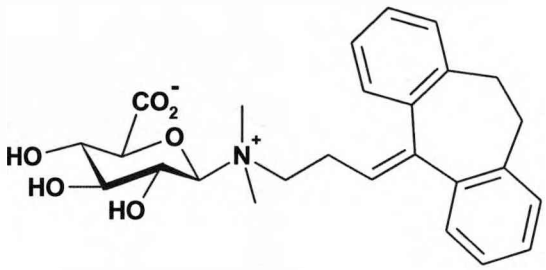
^1H NMR δ_H d_6-acetone: 1.08-1.44 (4H, m, $C\underline{H}_2CHC\underline{H}_2$), 1.8-1.97 (1H, m, $C\underline{H}CH_2OR$), 1.9-2.05 (9H, 3 x s, $COC\underline{H}_3$), 2.45-2.52 (1H, m $C\underline{H}_2NCH_2$), 2.86-2.95 (2H, m, $C\underline{H}_2NC\underline{H}_2$),

3.24-3.3 (1H, m, $CH_2NC\underline{H}_2$), 3.71 (3H, s, $CO_2C\underline{H}_3$), 4.02-4.19 (8H, 2 x s, m, aglycone), 4.22-4.25 (1H, d, 5-H, J = 10 Hz), 4.4-4.45 (1H, d, 1-H, J = 9.4 Hz), 5.03-5.08 (1H, t, 4-H, J = 9.7 Hz), 5.16-5.21 (1H, t, 2-H, J = 9.5 Hz), 5.32-5.38 (1H, t, 3-H, J = 9.6 Hz), 7.35-8.5 (6H, aglycone)

^{13}C NMR δ_C d_6-acetone: 14.9, 20.9, 21.1, 21.3, 37.1, 39.6, 45.1, 52.8, 53.2, 57.4, 60.9, 68.6, 71.3, 73.9, 74.8, 75.3, 94.9, 100.3, 106.2, 110.7, 120.3, 120.6, 128.8, 129.8, 137.1, 151.7, 152.0, 156.4, 157.2, 158.6, 169.1, 170.3, 170.5. ES (+ve ion mode) *m/z* $[M]^+$ 791 100 %.

Deprotection of the isobutyryl groups

β-*D-Glucopyranuronate-(N, N-dimethyl-3-(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-ylidene)-1-propanammonium) (4.133)*



To a solution of **(4.130)** (115 mg, 0.14 mmol) in MeOH (2 ml) was added 1M NaOH (0.42 ml, 0.42 mmol) at -10°C. The reaction was left stirring at -10°C for 3hrs at which time TLC (5:3:2 EtOAc/IPA/H_2O) showed completion. The reaction was then quenched with Amberlite IR-120 H^+ resin (140 mg, 0.42 mmol) till pH 7 was reached. The resin was then filtered and washed with methanol, and the methanol removed *in vacuo*. The residue was then partitioned between DCM (30 ml) and water (30 ml). The aqueous phase was separated and extracted with more DCM (3 x 30 ml) and then the water removed *in vacuo* to yield an amphorous glass (yield 76 %). The residue was then purified further using reverse phase C_{18} silica gel, eluting with 100 % H_2O, 10 % MeCN/H_2O to 25 % MeCN/H_2O.

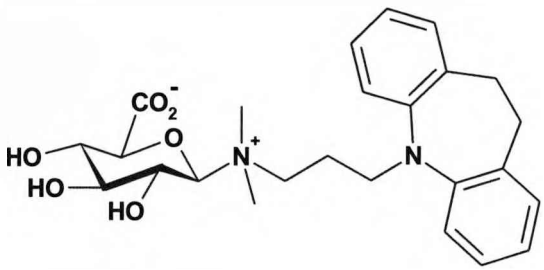
Yield: 16 %

Found: $[M]^+$ *m/z* 454.2227, $C_{26}H_{32}O_6N$ $[M]^+$ requires *m/z* 454.2230

^1H NMR δ_H MeOD 600 MHz @ 323K: 2.57-2.72 (2H, m, NCH_2CH_2CH), 2.78-3.0 (2H, broad m, $ArCH_2CH_2Ar$), 3.15 (3H, s, NCH_3), 3.19 (3H, s, NCH_3), 3.40-3.47 (3H, m, 3-H, NCH_2, $ArCH_2CH_2Ar$), 3.51-3.62 (2H, broad s, NCH_2, $ArCH_2CH_2Ar$), 3.69-3.74 (2H, m, 2-H, 4-H), 3.88-3.98 (1H, broad s, 5-H), 4.3-4.51 (1H, broad s, 1-H), 5.73-5.82 (1H, t, $=CHCH_2$), 7.04-7.31 (8H, m, ArH).

^{13}C NMR δ_C MeOD @323K: 24.4, 33.2, 34.9, 64.2, 64.8, 71.5, 71.6, 77.8, 78.7, 79.0, 79.1, 94.7, 95.2, 124.9, 125.2, 127.3, 127.7, 128.8, 131.1, 131.4, 138.5, 140.7, 140.8, 141.6, 148.5, 175.1.

β-D-glucopyranuronate-(N, N-dimethyl-3-(10,11-dihydro-5H-dibenzo[b,f]azepin-5-yl)-1-propanammmonium) (4.134)



To a solution of **(4.131)** (116 mg, 0.14 mmol) in MeOH (2 ml) was added 1M LiOH (0.42 ml, 0.42 mmol) at -10°C. The reaction was left stirring at -10°C for 2hrs at which time TLC (100 % EtOAc) showed completion. The reaction was then quenched with Amberlite H^+ resin (142 mg, 0.42 mmol) till pH 7 was reached. The resin was then filtered and washed with methanol, and the methanol removed *in vacuo*. The residue was then partitioned between DCM (30 ml) and water (30 ml). The aqueous phase was separated and extracted with more DCM (3 x 30 ml) and then the water removed *in vacuo* to yield an amorphous glass (yield 82 %). The residue was then purified further using preparative HPLC using neutral $MeCN/H_2O$.

Yield: 57 %

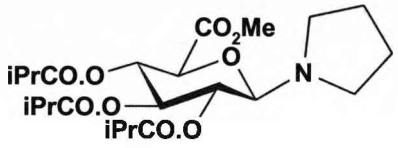
$[\alpha]^{293}$ = -18.25 (2 gml^{-1} in MeOH). Found: C, 60.97, H, 6.98, N, 5.37, $[M]^+$ *m/z* 457.2343. $C_{25}H_{32}O_6N_2.2H_2O$ requires C, 60.96, H, 7.36, N, 5.68 %; $C_{25}H_{33}O_6N_2$ requires *m/z* 457.2339

^{1}H NMR δ_H MeOD: 2.0-2.16 (2H, m, $N^+CH_2C\underline{H}_2CH_2$), 3.05 (3H, s, $NC\underline{H}_3$), 3.09 (3H, s, $NC\underline{H}_3$), 3.18 (4H, s, $C\underline{H}_2C\underline{H}_2$), 3.37-3.54 (3H, m, 3-H, $N^+CH_2CH_2C\underline{H}_2$), 3.69-3.71 (2H, m, 2-H, 4-H), 3.73-3.84 (2H, m, 5-H, $N^+C\underline{H}_2CH_2CH_2$), 3.88-3.98 (1H, m, $N^+C\underline{H}_2CH_2CH_2$), 4.51-4.55 (1H, d, 1-H, J = 8.9 Hz), 6.92-6.97 (2H, m, Ar$\underline{H}$), 7.11-7.20 (6H, m, Ar$\underline{H}$).

^{13}C NMR δ_C MeOD: 22.4, 33.6, (3 x peaks suspected to be amongst MeOD 48.6-50.1), 63.8, 71.9, 73.1, 78.9, 79.5, 95.8, 121.1, 124.5, 128.1, 131.5, 136.0, 149.5, 175.3. *m/z* (ES +ve ion mode) 457 $[M]^+$ 100 %.

Model compound

Methyl(N-(2,3,4-tri-O-isobutyryl)-β-D-glucopyranuronate)-pyrrolidine (4.139)



To a solution of **(4.129)** (1.09 g, 2.6 mmol) in toluene (8 ml) under a nitrogen atmosphere was added pyrrolidine (0.24 ml, 3.12 mmol). The reaction was heated to 50°C for 6 hrs. The toluene and excess pyrrolidine were removed *in vacuo*.

Yield: 100 %

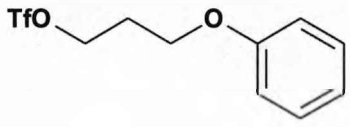
Found $[M+H]^+$ *m/z* 472.256 $C_{23}H_{38}O_9N$ requires *m/z* 472.255

^{1}H NMR δ_H $CDCl_3$: 0.98-1.2 (18H, m, $CO_2CH(C\underline{H}_3)_2$), 1.6-1.61 (4H, m, $C\underline{H}_2C\underline{H}_2$), 2.28-2.42 (3H, m, $CO_2C\underline{H}(CH_3)_2$), 2.68-2.84 (4H, m, $C\underline{H}_2NC\underline{H}_2$), 3.67 (3H, s, $CO_2C\underline{H}_3$), 3.9-3.93 (1H, d, 5-H, J = 10.0 Hz), 4.28-4.31 (1H, d, 1-H, J = 9.3 Hz), 5.03-5.14 (2H, 2 x t, 2-H, J = 9.4 Hz, 4-H, J = 9.8 Hz), 5.2-5.26 (1H, t, 3-H, J = 9.5 Hz)

^{13}C NMR δ_C $CDCl_3$: 17.7, 17.8, 17.9, 23.5, 32.8, 32.9, 32.9, 45.7, 51.5, 67.9, 68.7, 71.7, 72.9, 89.1, 174.3, 174.5, 174.9, 181.5. *m/z* (ES +ve ion mode) 472 $[M+H]^+$ 100 %.

-Synthesis of trifluoromethane sulfonate activated electrophiles

(3-Phenoxypropyl) trifluoromethane sulfonate (4.140)



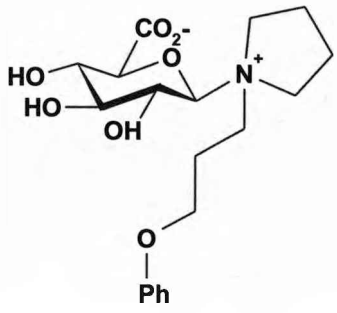
To a solution of 3-phenoxypropan-1-ol (1 ml, 7 mmol) in DCM (3 ml) at 0°C under N_2 was added 2,3,6-collidine (1 ml, 7.7 mmol) followed by triflic anhydride (1.29 ml, 7.7 mmol). The reaction was left at 0°C for 2 hrs and then the solvent removed *in vacuo*. The residue was dissolved with diethylether (50 ml) and water (30 ml) and the organic layer separated. The organic layer was then successively washed with 1M HCl (2 X 20 ml), sat. $NaHCO_3$ (20 ml), water (30 ml) and then dried over $MgSO_4$. This material was used without further purification.

Yield: 30 %

^1H NMR δ_H $CDCl_3$ 250 MHz: 2.22-2.40 (2H, q, $CH_2\underline{CH_2}CH_2$), 4.0-4.18 (2H, t, $CH_2\underline{CH_2}$-OAr), 4.68-4.90 (2H, t, $TfO\underline{CH_2}$), 6.82-7.10 (3H, m, ArH), 7.26-7.41 (2H, m, ArH).

- Quaternisation of the pyrrolidine sugar with 3-phenoxypropyl trifluoromethane sulfonate, followed by deprotection

β-D-glucopyranuronate-N-1-(3-phenoxypropyl)pyrrolidinium-1-yl (4.141)



To a solution of **(4.139)** (740 mg, 1.57 mmol) in dry DCE (15 ml) under nitrogen was added **(4.140)** (488 mg, 1.72 mmol) in DCE (5 ml). The reaction was heated to 50°C for 24 hrs. The DCE was then removed *in vacuo* and the residue triturated with diethyl ether. Some of the residue (100 mg, 0.13 mmol) was then dissolved in methanol (8 ml) and cooled to -10°C. To the stirring solution was added 10 % aq. Na_2CO_3 (0.56 ml, 0.52 mmol). After addition the reaction was left stirring at -10°C for 2 hrs. Amberlite IR-120 H^+ ion exchange resin was then added to the stirring solution until pH 6-7 was achieved. The methanol was then removed *in vacuo* and the residue partitioned between water (50 ml) and DCM (50 ml). The aqueous phase was then extracted with DCM (3 X 50 ml) and the aqueous phase was then evaporated to dryness to give an amphorous solid. NMR showed that this material was 4:1 product/eliminated by-product
Yield: 61 % product.

HPLC was used to purify the material further; yield after HPLC 11 %

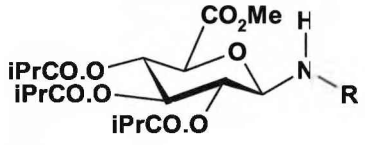
$[\alpha]^{293}$ = -11.47 (0.8 gml^{-1} in MeOH). Found: $[M]^+$ *m/z* 382.1871; $C_{19}H_{28}O_7N$ requires *m/z* 382.1866

^{1}H NMR δ_H MeOD 600MHz: 2.08-2.27 (4H, m, $CH_2\underline{CH_2}\underline{CH_2}CH_2$), 2.28-2.46 (2H, m, $NCH_2\underline{CH_2}CH_2O$), 3.46-3.53 (2H, m, 3-H, 4-H), 3.61-3.69 (1H, m, $\underline{CH_2}NCH_2$), 3.71-3.79 (4H, m, $CH_2N\underline{CH_2}$, $NCH_2CH_2\underline{CH_2}O$, 5-H), 3.79-3.83 (1H, t, 2-H, J = 8.9 Hz), 3.92-4.11 (4H, m, $N\underline{CH_2}CH_2CH_2O$, $CH_2N\underline{CH_2}$), 4.67-4.71 (1H, d, 1-H, J = 8.9 Hz), 6.89-6.93 (3H, m, Ar<u>H</u>), 7.22-7.28 (2H, m, <u>ArH</u>).

^{13}C NMR δ_C MeOD: 23.5, 24.8, 25.6, 59.3, 62.4, 64.6, 66.2, 72.2, 73.2, 79.1, 79.8, 96.3, 115.9, 122.6, 130.9, 160.4, 175.4. *m/z* (ES +ve ion mode) 382 $[M]^+$ 100 %.

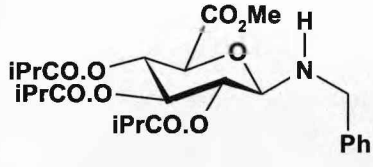
-Formation of a pyrrolidine ring by intramolecular quaternisation

-The condensation reaction



To a solution of hemiacetal **(4.129)** (2 g, 5 mmol) in toluene (15 ml) was added the amine (5 mmol) at 50°C. The reaction was left heating for 17 hrs and then allowed to cool and the solvent removed *in vacuo*.

Methyl ((2,3,4-tri-O-isobutyryl)-β-D-1-N-benzylamino)-glucopyranuronate (4.144)



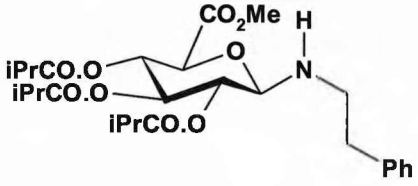
Yield: ~95 % (taken from NMR integration), used without any further purification

Found $[M+H]^+$ *m/z* 508.252; $C_{26}H_{38}NO_9$ requires *m/z* 508.254.

^{1}H NMR δ_H $CDCl_3$: 0.99-1.12 (18H, m, $(C\underline{H_3})_2CH$), 2.32-2.49 (3H, m, $(CH_3)_2\underline{CH}$), 3.68 (3H, s, $CO_2\underline{CH_3}$), 3.78-3.82 (1H, d, $\underline{CH_2}Ar$), 3.89-3.92 (1H, d, 5-H, J = 10.0 Hz), 3.97-4.0

(1H, d, C$\underline{H}_2$Ar), 4.05-4.07 (1H, d, 1-H, J = 9.12 Hz), 4.88-4.91 (1H, t, 2-H, J = 9.2 Hz), 5.06-5.11 (1H, t, 4-H, J = 9.8 Hz), 5.21-5.26 (1H, t, 3-H, J = 9.6 Hz), 7.15-7.22 (5H, m, ArH).

Methyl(2,3,4-tri-O-isobutyryl)-1-N-ethylphenylamino -β-D-glucopyranuronate (4.147)



5:2 product/hemiacetal sugar (taken from NMR intergration). Used without further purification.

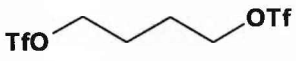
Found: $[M+Na]^+$ *m/z* 544.254, $C_{27}H_{39}O_9NNa$ requires *m/z* 544.254

^{1}H NMR δ_H $CDCl_3$: 0.99-1.18 (18H, m, $(C\underline{H}_3)_2CH$), 2.32-2.50 (3H, m, $(CH_3)_2C\underline{H}$), 2.61-3.0 (3H, m, $C\underline{H}_2C\underline{H}_2Ar$), 3.08-3.17 (1H, dt, $NC\underline{H}_2CH_2$, J = 11.9, 7.0 Hz), 3.64 (3H, s, CO_2-$C\underline{H}_3$), 3.89-3.92 (1H, d, 5-H, J = 9.9 Hz), 4.06-4.09 (1H, d, 1-H, J = 9.1 Hz), 4.78-4.82 (1H, t, 2-H, J = 9.3 Hz), 5.04-5.09 (1H, t, 4-H, J = 9.9 Hz), 5.24-5.29 (1H, t, 3-H, J = 9.7 Hz), 7.03-7.23 (5H, m, Ar$\underline{H}$).

^{13}C NMR δ_C $CDCl_3$: 17.6, 17.7, 17.9, 32.8 (x 2), 32.9, 35.9, 45.9, 51.7, 68.8, 69.2, 70.9, 72.6, 88.8, 127.2, 127.3, 127.7, 128.0, 174.4, 174.9, 175.0, 181.1.

Forming the alkylating agent

Butane-1,4-diyl bis(trifluoromethanesulfonate) (4.145) [22].



To a solution of trifluoromethanesulfonic anhydride (4.95 g, 0.015 mmol) in DCM (50 ml) was added dropwise THF (1.08 g, 0.015 mmol) in DCM (50 ml) at -78°C. On complete addition the reaction was allowed to warm to room temperature (~1hr). The reaction mixture was then washed with water (100 ml) and dried over Na_2SO_4. A re-crystallisation was achieved using diethyl ether

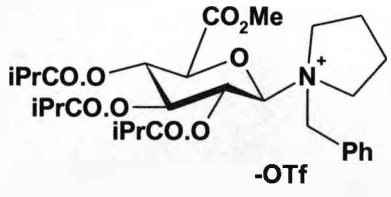
Yield: 71 %

^{1}H NMR δ_H $CDCl_3$: 2.01 (4H, m, $C\underline{H}_2CH_2$), 4.60 (4H, m, $C\underline{H}_2OSO_2CF_3$)

^{13}C NMR δ_C $CDCl_3$: 25.72, 76.30, 114.21, 117.39, 120.58, 123.75.

-Alkylation, then intramolecular quaternisation

Methyl((2,3,4-tri-O-isobutyryl)-β-D-glucopyranuronate)-N-pyrrolidinium-1-benzyl trifluoromethane sulfonate (4.146)



To a solution of **(4.144)** (379 mg, 0.75 mmol) and 2,6-lutidine (0.095 ml, 0.83 mmol) in DCE (4 ml) at 50°C was added **(4.145)** (291mg, 0.83 mmol) in a solution of DCE (4 ml) dropwise. The reaction was left heating at 50°C for 24 hrs, and the DCE removed *in vacuo*. The residue was filtered through a pad of silica (7 cm depth, 5cm diameter) with EtOAc, then 10 % EtOH/DCM. The solvent was removed *in vacuo*.

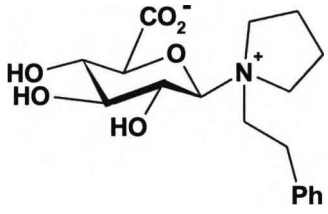
NMR showed some remaining impurities. Further purification on silica gel lead to hydrolysis.

Yield: 27 %, purity ~ 80 %

^{1}H NMR δ_H CD_3CN: 1.03-1.12 (18H, m, 3 x $CO_2CH(CH_3)_2$), 1.65-1.88 (2H, m, CH_2CH_2), 1.94-2.11 (2H, m, CH_2CH_2), 2.41-2.52 (2H, sept, 2 x $CO_2CH(CH_3)_2$), 2.57-2.68 (1H, sept, $CO_2CH(CH_3)_2$), 3.37-3.42 (1H, m, CH_2NCH_2), 3.51-3.58 (1H, m, CH_2NCH_2), 3.71 (3H, s, CO_2CH_3), 3.72-3.80 (2H, m, CH_2NCH_2), 4.49-4.53 (2H, 2 x d, 5-H, J = 9.7 Hz, CH_2Ar, J = 13.2 Hz), 4.62-4.65 (1H, d, CH_2Ar, J = 13.2 Hz), 5.12-5.15 (1H, d, 1-H J = 8.96 Hz), 5.28 (1H, t, 3-H, J = 9.3 Hz), 5.52 (1H, t, 4-H, J = 9.3 Hz), 5.71 (1H, t, 2-H, J = 8.8 Hz), 7.52-7.68 (5H, m, ArH).

-Alkylation, then intramolecular quaternisation and deprotection

β-D-glucopyranuronate-N-1-(2-phenylethyl)pyrrolidinium-1-yl (4.150)



To a solution of **(4.147)** (1.37 g, 2.72 mmol) in DCE (8 ml) was added 2,6-lutidine (0.63 ml, 5.44 mmol). The solution was evacuated with N_2 and heated to 50°C. The bistriflate, **(4.145)** (1.45 g, 4.08 mmol) was then added in DCE (8 ml) to the stirring reaction. The reaction was left at 50°C for 18 hrs. The solvent was then removed *in vacuo* and the residue filtered through a pad of silica, using 100 % EtOAc, then 10 % IPA/DCM with the product eluting in the latter. The solvent was removed *in vacuo* and the residue triturated with diethyl ether. The residue (313 mg, 0.44 mmol) was then dissolved in MeOH (6 ml) and cooled to -10°C. A solution of 10 % Na_2CO_3 (aq) (1.87 ml, 1.76 mmol) was added drop wise. The reaction was then left at -10°C for 2hrs and RT for 1h. The reaction was quenched with the addition of Amberlite IR-

120 H^+ ion exchange resin until pH 6-7 was reached. The solvent was then removed *in vacuo* and the residue partitioned between DCM (20 ml) and water (30 ml). The water layer was extracted with DCM (3 x 10 ml) and then the water removed *in vacuo* to yield a yellow viscous oil. The residue was then purified by reverse phase preparative TLC using 20 % MeOH/H_2O. The reverse phase silica gel was washed with MeOH to liberate the product.

Yield: 10 %, purity ~ 60 %, the NMR spectrum suggests some hydrolysis of the aglycone.

Found: $[M+H]^+$ *m/z* 352.176. $C_{18}H_{26}O_6N$ requires *m/z* 352.176

^1H NMR δ_H d_6-acetone: 2.11-2.15 (4H, m, $C\underline{H}_2C\underline{H}_2$), 3.14-3.25 (2H, m, $C\underline{H}_2Ph$), 3.61-3.78 (2H, 2 x t 3-H J = 8.6 Hz, 4-H, J = 9.2 Hz), 3.79-3.89 (1H, m, $NC\underline{H}_2CH_2Ph$), 3.90-4.13 (2H, t and d, 2-H J = 8.6 Hz, 5-H, J = 9.3 Hz), 4.05-4.22 (1H, m, $NC\underline{H}_2CH_2Ph$), 4.68-4.79 (4H, m, $C\underline{H}_2NC\underline{H}_2$), 5.0-5.03 (1H, d, 1-H, J = 9 Hz), 7.21-7.5 (5H, m, Ar$\underline{H}$).

^{13}C NMR δ_C d_6-acetone: 23.8, 24.2, 49.9, 53.5, 61.9, 64.69, 71.8, 72.4, 78.1, 79.0, 94.8, 129.5, 130.1, 130.5, 145.9, 156.9.

5.4 References

(1) Bowkett, E. R.; Harding, J. R.; Maggs, J. L.; Park, B. K.; Perrie, J. A.; Stachulski, A. V. *Tetrahedron* **2007**, *63*, 7596-7605.

(2) Perrie, J. A.; Harding, J. R.; Holt, D. W.; Johnston, A.; Meath, P.; Stachulski, A. V. *Organic Letters* **2005**, *7*, 2591-2594.

(3) Chen, F. M. F.; Lee, Y.; Steinauer, R.; Benoiton, N. L. *Canadian Journal of Chemistry-Revue Canadienne De Chimie* **1987**, *65*, 613-618.

(4) Mukaiyama, T.; Tanaka, T. *Chemistry Letters* **1976**, 303-306.

(5) Kokotos, G.; Noula, C. *Journal of Organic Chemistry* **1996**, *61*, 6994-6996.

(6) Chaudhary, S. K.; Hernandez, O. *Tetrahedron Letters* **1979**, 95-98.

(7) Bollenback, G. N.; Long, J. W.; Benjamin, D. G.; Lindquist, J. A. *Journal of the American Chemical Society* **1955**, *77*, 3310-3315.

(8) Brown, R. T.; Carter, N. E.; Mayalarp, S. P.; Scheinmann, F. *Tetrahedron* **2000**, *56*, 7591-7594.

(9) Bickley, J.; Cottrell, J. A.; Ferguson, J. R.; Field, R. A.; Harding, J. R.; Hughes, D. L.; Kartha, K. P. R.; Law, J. L.; Scheinmann, F.; Stachulski, A. V. *Chemical Communications* **2003**, 1266-1267.

(10) Koike, K.; Sugimoto, M.; Sato, S.; Ito, Y.; Nakahara, Y.; Ogawa, T. *Carbohydrate Research* **1987**, *163*, 189-208.

(11) Granata, A.; Perlin, A. S. *Carbohydrate Research* **1980**, *86*, 305-308.

(12) Tietze, L. F.; Seele, R. *Carbohydrate Research* **1986**, *148*, 349-352.

(13) Excoffier, G.; Gagnaire, D.; Utille, J. P. *Carbohydrate Research* **1975**, *39*, 368-373.

(14) Leroux, J.; Perlin, A. S. *Carbohydrate Research* **1978**, *67*, 163-178.

(15) Baker *J. Chem. Soc.* **1929**, 1206.

(16) Pitt, N.; Duane, R. M.; O' Brien, A.; Bradley, H.; Wilson, S. J.; O'Boyle, K. M.; Murphy, P. V. *Carbohydrate Research* **2004**, *339*, 1873-1887.

(17) Sabesan, S.; Neira, S. *Carbohydrate Research* **1992**, *223*, 169-185.

(18) Kishikaw.T; Yuki, H. *Chemical & Pharmaceutical Bulletin* **1966**, *14*, 1360-&.

(19) Perrin, C. L.; Armstrong, K. B. *Journal of the American Chemical Society* **1993**, *115*, 6825-6834.

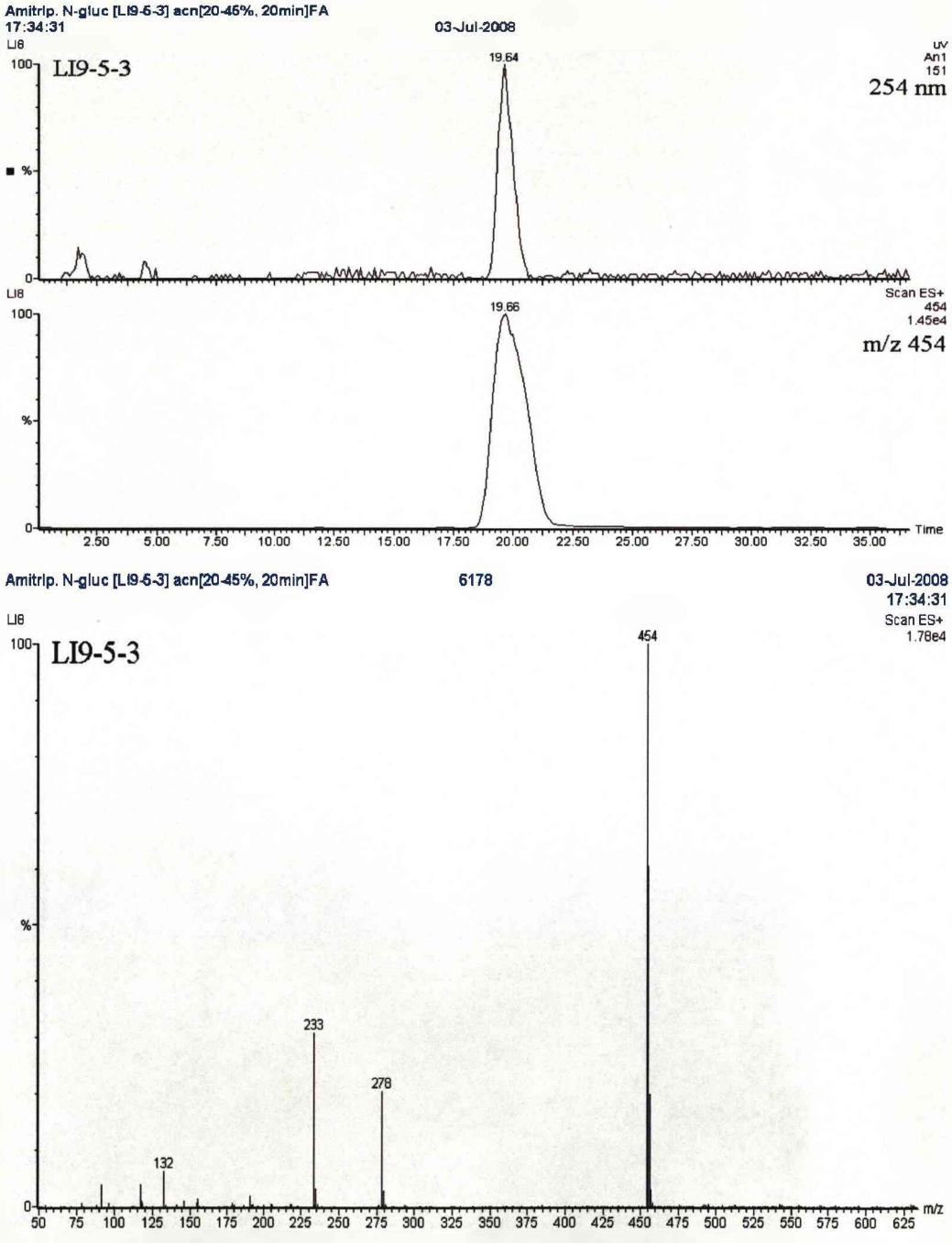
(20) Hodge, J. E.; Rist, C. E. *Journal of the American Chemical Society* **1952**, *74*, 1498-1500.

(21) Galgali, P.; Agashe, M.; Varma, A. J. *Carbohydrate Polymers* **2007**, *67*, 576-585.

(22) Beard, C. D.; Baum, K.; Grakausk.V *Journal of Organic Chemistry* **1973**, *38*, 3673-3677.

6.1 Appendix

6.1.1 LCMS data of Amitriptyline N^+-Glucuronide (4.133)



6.1.2 Crystallographic data for *2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl(trimethylammonium) iodide (4.96)*

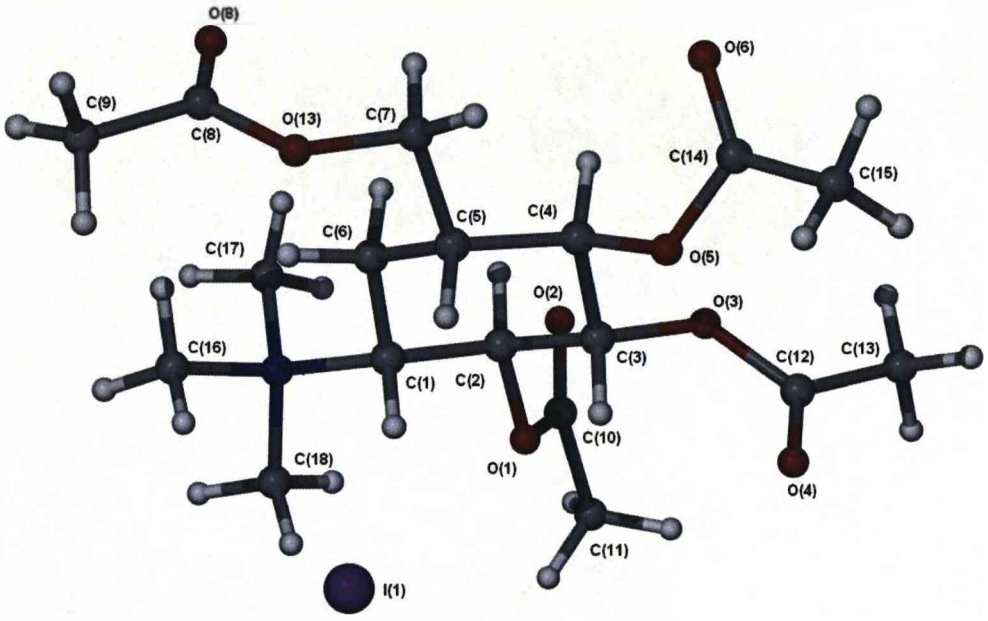


Table 1. Crystal data and structure refinement for **(4.96)**

Identification code	LI733	
Empirical formula	C17 H28 I N O9	
Formula weight	517.30	
Temperature	173(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	P212121	
Unit cell dimensions	a = 8.616(2) Å	α= 90°.
	b = 11.244(2) Å	β= 90°.
	c = 24.390(5) Å	γ = 90°.
Volume	2362.9(8) $Å^3$	
Z	4	
Density (calculated)	1.454 Mg/m^3	
Absorption coefficient	1.397 mm^{-1}	
F(000)	1048	
Crystal size	0.44 x 0.42 x 0.29 mm^3	
Theta range for data collection	1.99 to 24.18°.	
Index ranges	-9<=h<=9, -12<=k<=12, -27<=l<=27	
Reflections collected	18276	
Independent reflections	3557 [R(int) = 0.0456]	
Completeness to theta = 24.18°	94.2 %	
Absorption correction	Numerical	
Max. and min. transmission	0.6874 and 0.5784	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3557 / 0 / 260	
Goodness-of-fit on F^2	0.854	

Final R indices [I>2sigma(I)]	R1 = 0.0274, wR2 = 0.0519
R indices (all data)	R1 = 0.0392, wR2 = 0.0544
Absolute structure parameter	-0.030(17)
Largest diff. peak and hole	0.465 and -0.397 e.Å^{-3}

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3)

for **(4.96)**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
I(1)	2070(1)	7812(1)	2283(1)	68(1)
O(6)	2226(4)	6046(2)	4243(1)	51(1)
O(4)	5395(3)	5436(2)	4105(1)	48(1)
C(5)	2747(5)	8064(3)	3950(2)	46(1)
N(1)	5975(5)	9405(3)	3216(1)	51(1)
O(1)	4026(3)	8834(2)	3813(1)	47(1)
O(2)	6989(4)	6775(2)	3338(1)	55(1)
C(1)	5024(5)	8349(3)	3417(2)	43(1)
O(9)	2706(5)	11022(3)	4448(1)	71(1)
O(5)	4068(5)	4124(3)	3577(2)	75(1)
O(8)	1083(4)	9761(3)	4041(1)	67(1)
C(2)	5878(4)	7340(3)	3693(1)	42(1)
C(4)	3408(4)	6931(3)	4205(2)	40(1)
C(3)	4643(5)	6412(3)	3834(2)	40(1)
C(7)	1713(6)	8757(4)	4327(2)	56(1)
C(17)	7006(7)	9894(4)	3654(2)	71(1)
C(10)	8419(6)	6544(4)	3560(3)	68(2)
C(8)	1746(6)	10834(4)	4124(2)	65(1)
C(16)	4857(6)	10363(4)	3030(2)	79(2)
O(3)	8786(5)	6904(4)	3997(2)	118(2)
C(18)	6916(7)	9063(4)	2729(2)	83(1)
C(12)	4981(6)	4305(4)	3935(2)	54(1)

C(13)	5882(8)	3388(4)	4246(2)	81(2)
O(7)	1890(9)	6345(5)	5118(2)	176(3)
C(14)	1677(9)	5741(5)	4726(2)	103(2)
C(11)	9405(7)	5861(5)	3173(3)	99(2)
C(15)	582(8)	4724(5)	4705(3)	108(2)
C(9)	1035(8)	11759(5)	3760(3)	108(2)

Table 3. Bond lengths [Å] and angles [°] for **(4.96)**.

O(6)-C(14)	1.313(6)
O(6)-C(4)	1.427(4)
O(4)-C(12)	1.384(5)
O(4)-C(3)	1.435(5)
C(5)-O(1)	1.440(5)
C(5)-C(7)	1.499(6)
C(5)-C(4)	1.529(5)
N(1)-C(18)	1.489(6)
N(1)-C(17)	1.495(5)
N(1)-C(16)	1.514(6)
N(1)-C(1)	1.523(5)
O(1)-C(1)	1.403(5)
O(2)-C(10)	1.371(6)
O(2)-C(2)	1.439(5)
C(1)-C(2)	1.511(5)
O(9)-C(8)	1.165(5)
O(5)-C(12)	1.192(5)
O(8)-C(8)	1.350(6)
O(8)-C(7)	1.435(5)
C(2)-C(3)	1.530(5)
C(4)-C(3)	1.513(5)
C(10)-O(3)	1.183(6)
C(10)-C(11)	1.485(7)
C(8)-C(9)	1.498(7)
C(12)-C(13)	1.497(7)
O(7)-C(14)	1.187(6)

C(14)-C(15)	1.483(8)
C(14)-O(6)-C(4)	119.8(4)
C(12)-O(4)-C(3)	116.7(3)
O(1)-C(5)-C(7)	106.5(3)
O(1)-C(5)-C(4)	108.1(3)
C(7)-C(5)-C(4)	113.8(3)
C(18)-N(1)-C(17)	110.0(4)
C(18)-N(1)-C(16)	106.9(4)
C(17)-N(1)-C(16)	109.3(4)
C(18)-N(1)-C(1)	110.4(3)
C(17)-N(1)-C(1)	112.1(3)
C(16)-N(1)-C(1)	107.9(4)
C(1)-O(1)-C(5)	113.2(3)
C(10)-O(2)-C(2)	116.4(3)
O(1)-C(1)-C(2)	106.5(3)
O(1)-C(1)-N(1)	104.4(3)
C(2)-C(1)-N(1)	117.8(4)
C(8)-O(8)-C(7)	118.0(4)
O(2)-C(2)-C(1)	112.9(3)
O(2)-C(2)-C(3)	107.3(3)
C(1)-C(2)-C(3)	105.9(3)
O(6)-C(4)-C(3)	105.8(3)
O(6)-C(4)-C(5)	110.0(3)
C(3)-C(4)-C(5)	109.9(3)
O(4)-C(3)-C(4)	109.7(3)
O(4)-C(3)-C(2)	108.1(3)
C(4)-C(3)-C(2)	111.1(3)
O(8)-C(7)-C(5)	109.5(3)

O(3)-C(10)-O(2)	122.1(5)
O(3)-C(10)-C(11)	126.7(6)
O(2)-C(10)-C(11)	111.1(5)
O(9)-C(8)-O(8)	124.4(4)
O(9)-C(8)-C(9)	124.5(5)
O(8)-C(8)-C(9)	111.0(5)
O(5)-C(12)-O(4)	123.1(4)
O(5)-C(12)-C(13)	126.5(4)
O(4)-C(12)-C(13)	110.4(4)
O(7)-C(14)-O(6)	121.1(5)
O(7)-C(14)-C(15)	124.5(5)
O(6)-C(14)-C(15)	113.5(5)

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$)for li733. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2 a^{*2} U^{11} + ... + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
I(1)	73(1)	58(1)	72(1)	-16(1)	-26(1)	9(1)
O(6)	44(2)	54(2)	55(2)	2(1)	11(1)	-10(2)
O(4)	50(2)	44(2)	48(2)	3(1)	-3(1)	-7(1)
C(5)	38(3)	50(2)	51(2)	2(2)	-4(2)	-10(2)
N(1)	54(3)	55(2)	44(2)	15(2)	-7(2)	-23(2)
O(1)	45(2)	44(2)	52(2)	4(1)	-4(1)	-10(1)
O(2)	50(2)	57(2)	57(2)	-7(1)	14(2)	-17(2)
C(1)	41(3)	49(2)	39(2)	1(2)	-9(2)	-17(2)
O(9)	71(3)	63(2)	79(2)	-5(2)	-29(2)	-1(2)
O(5)	90(3)	60(2)	75(2)	-9(2)	-22(2)	-20(2)
O(8)	55(2)	55(2)	92(2)	0(2)	-22(2)	0(2)
C(2)	38(3)	47(2)	41(2)	1(2)	1(2)	-14(2)
C(4)	38(3)	39(2)	44(2)	2(2)	3(2)	-10(2)
C(3)	38(3)	40(2)	42(2)	2(2)	-2(2)	-6(2)
C(7)	46(4)	54(3)	67(3)	-1(2)	-6(2)	0(2)
C(17)	76(4)	73(3)	65(3)	20(2)	-23(3)	-41(3)
C(10)	27(4)	67(3)	110(4)	-21(3)	7(3)	-13(2)
C(8)	59(4)	58(3)	79(3)	-3(2)	-18(3)	6(3)
C(16)	81(4)	59(3)	97(4)	32(3)	-29(3)	-15(3)
O(3)	60(3)	130(4)	163(4)	-77(3)	-46(3)	27(2)
C(18)	101(4)	83(3)	65(3)	17(3)	20(4)	-23(3)
C(12)	64(4)	46(3)	52(3)	-1(2)	8(2)	-6(2)

C(13)	114(5)	48(3)	81(4)	6(3)	-3(3)	4(3)
O(7)	283(8)	177(5)	68(3)	-12(3)	54(4)	-121(5)
C(14)	158(7)	93(4)	57(3)	-13(3)	43(4)	-50(4)
C(11)	49(4)	105(4)	142(6)	-38(4)	27(3)	-10(3)
C(15)	115(6)	88(4)	120(5)	3(4)	59(4)	-46(4)
C(9)	104(5)	68(4)	153(6)	14(4)	-73(4)	8(3)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for **(4.96)**.

	x	y	z	U(eq)
H(5)	2162	7857	3608	56
H(1)	4394	8027	3106	52
H(2)	6399	7629	4034	51
H(4)	3850	7104	4576	48
H(3)	4147	6121	3489	48
H(7A)	859	8242	4460	67
H(7B)	2314	9032	4649	67
H(17A)	7817	9313	3741	107
H(17B)	6390	10057	3984	107
H(17C)	7486	10633	3525	107
H(16A)	4327	10700	3350	119
H(16B)	4089	10017	2780	119
H(16C)	5432	10992	2840	119
H(18A)	7451	9766	2585	124
H(18B)	6232	8735	2445	124
H(18C)	7683	8462	2835	124
H(13A)	5206	2712	4331	121
H(13B)	6273	3736	4587	121
H(13C)	6758	3116	4022	121
H(11A)	10359	5610	3359	148
H(11B)	9670	6363	2858	148
H(11C)	8838	5158	3045	148

H(15A)	1160	3989	4636	162
H(15B)	-170	4852	4409	162
H(15C)	33	4661	5055	162
H(9A)	49	12026	3919	162
H(9B)	847	11420	3396	162
H(9C)	1743	12438	3728	162

Table 6. Torsion angles [°] for **(4.96)**

C(5)-O(1)-C(1)-N(1)	164.2(3)
C(5)-O(1)-C(1)-C(2)	-70.5(4)
C(1)-O(1)-C(5)-C(4)	63.2(4)
C(1)-O(1)-C(5)-C(7)	-174.2(3)
C(10)-O(2)-C(2)-C(1)	135.0(3)
C(10)-O(2)-C(2)-C(3)	-108.7(4)
C(2)-O(2)-C(10)-O(3)	-8.2(7)
C(2)-O(2)-C(10)-C(11)	174.6(4)
C(12)-O(4)-C(3)-C(2)	-134.8(3)
C(12)-O(4)-C(3)-C(4)	103.8(4)
C(3)-O(4)-C(12)-O(5)	1.2(6)
C(3)-O(4)-C(12)-C(13)	178.9(4)
C(14)-O(6)-C(4)-C(3)	131.4(4)
C(14)-O(6)-C(4)-C(5)	-110.0(5)
C(4)-O(6)-C(14)-O(7)	16.1(9)
C(4)-O(6)-C(14)-C(15)	-173.8(4)
C(8)-O(8)-C(7)-C(5)	-100.3(5)
C(7)-O(8)-C(8)-O(9)	-7.0(7)
C(7)-O(8)-C(8)-C(9)	175.3(4)
C(16)-N(1)-C(1)-O(1)	-54.4(4)
C(17)-N(1)-C(1)-O(1)	66.1(4)

C(18)-N(1)-C(1)-O(1)	-170.9(3)
C(16)-N(1)-C(1)-C(2)	-172.2(3)
C(17)-N(1)-C(1)-C(2)	-51.8(5)
C(18)-N(1)-C(1)-C(2)	71.3(4)
O(1)-C(1)-C(2)-O(2)	-178.2(3)
O(1)-C(1)-C(2)-C(3)	64.7(3)
N(1)-C(1)-C(2)-O(2)	-61.6(4)
N(1)-C(1)-C(2)-C(3)	-178.7(3)
O(2)-C(2)-C(3)-O(4)	59.9(3)
O(2)-C(2)-C(3)-C(4)	-179.5(3)
C(1)-C(2)-C(3)-O(4)	-179.3(3)
C(1)-C(2)-C(3)-C(4)	-58.8(4)
O(4)-C(3)-C(4)-O(6)	-68.4(4)
O(4)-C(3)-C(4)-C(5)	172.9(3)
C(2)-C(3)-C(4)-O(6)	172.0(3)
C(2)-C(3)-C(4)-C(5)	53.3(4)
O(6)-C(4)-C(5)-O(1)	-168.1(3)
O(6)-C(4)-C(5)-C(7)	73.8(4)
C(3)-C(4)-C(5)-O(1)	-52.0(4)
C(3)-C(4)-C(5)-C(7)	-170.1(3)
O(1)-C(5)-C(7)-O(8)	64.3(4)
C(4)-C(5)-C(7)-O(8)	-176.7(3)

Table 7. Hydrogen bonds for **(4.96)** [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
C(1)-H(1)...I(1)	1.0000	2.8500	3.806(4)	161.00
C(2)-H(2)...O(3)	1.0000	2.2100	2.659(6)	105.00
C(2)-H(2)...O(7)#1	1.0000	2.4100	3.371(6)	162.00
C(3)-H(3)...O(5)	1.0000	2.2600	2.694(5)	105.00
C(4)-H(4)...O(7)	1.0000	2.3100	2.665(6)	100.00
C(7)-H(7A)...O(3)#2	0.9900	2.5900	3.369(7)	135.00
C(7)-H(7B)...O(9)	0.9900	2.3200	2.703(6)	102.00
C(11)-H(11B)...I(1)#3	0.9800	2.9800	3.846(6)	148.00
C(11)-H(11C)...I(1)#4	0.9800	2.8700	3.822(6)	165.00
C(15)-H(15C)...O(9)#5	0.9800	2.4700	3.333(8)	148.00
C(16)-H(16C)...I(1)#6	0.9800	2.9800	3.896(5)	155.00
C(17)-H(17B)...O(1)	0.9800	2.4900	2.857(6)	102.00
C(18)-H(18B)...O(5)#6	0.9800	2.5400	3.298(6)	133.00
C(18)-H(18C)...O(2)	0.9800	2.3400	2.971(5)	122.00

Symmetry transformations used to generate equivalent atoms:

#1 2 #2+0 #3+2 #4 1/2-x+1,-y-1,1/2+z #5+1

#6 1/2-x+1,-y,1/2+z

6.1.3 Crystallographic data for β-*D-Glucopyranosyl(trimethylammonium) iodide (4.99)*

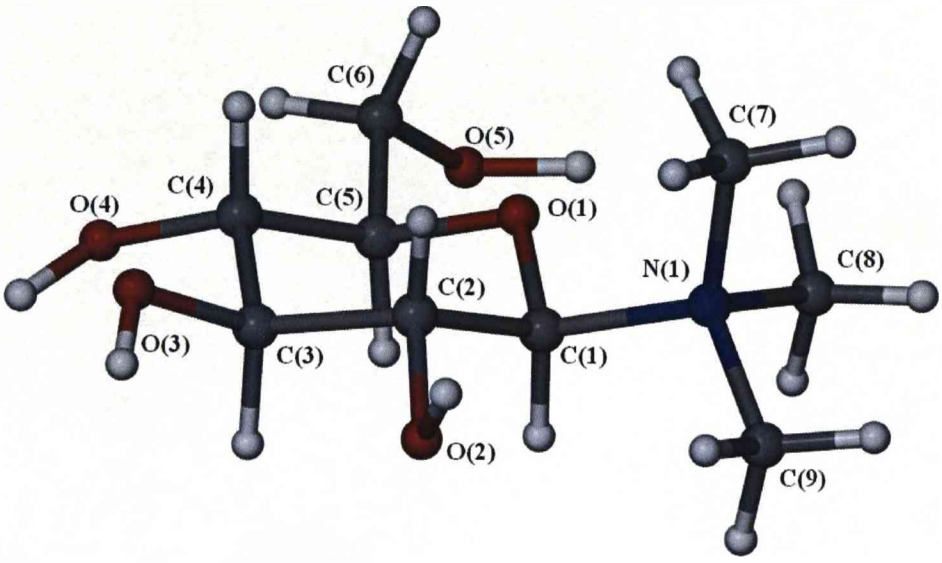


Table 1. Crystal data and structure refinement for **(4.99)**.

Identification code	ck348m	
Empirical formula	C9 H20 I N O5	
Formula weight	349.16	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	P212121	
Unit cell dimensions	a = 9.3032(19) Å	α= 90°.
	b = 9.985(2) Å	β = 90°.
	c = 14.663(3) Å	γ = 90°.
Volume	1362.0(5) $Å^3$	
Z	4	
Density (calculated)	1.703 Mg/m^3	
Absorption coefficient	2.358 mm^{-1}	
F(000)	696	
Crystal size	0.32 x 0.19 x 0.15 mm^3	
Theta range for data collection	2.47 to 27.51°.	
Index ranges	-12<=h<=11, -12<=k<=10, -14<=l<=18	
Reflections collected	8145	
Independent reflections	3038 [R(int) = 0.0177]	
Completeness to theta = 27.51°	99.0 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7187 and 0.5192	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3038 / 8 / 189	
Goodness-of-fit on F^2	1.026	
Final R indices [I>2sigma(I)]	R1 = 0.0182, wR2 = 0.0477	
R indices (all data)	R1 = 0.0184, wR2 = 0.0478	
Absolute structure parameter	0.02(2)	
Largest diff. peak and hole	0.738 and -0.347 $e.Å^{-3}$	

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for **(4.99)**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
O(1)	5656(2)	5478(2)	5276(1)	16(1)
O(2)	4010(2)	2150(2)	5293(1)	18(1)
O(3)	5633(2)	2244(2)	6957(1)	16(1)
O(4)	5858(2)	4878(2)	7727(1)	17(1)
O(5)	5874(3)	8243(2)	6184(1)	20(1)
N(1)	4745(2)	4484(2)	3966(2)	14(1)
C(1)	4667(3)	4486(3)	5003(2)	13(1)
C(2)	5053(3)	3155(3)	5463(2)	13(1)
C(3)	5071(3)	3385(3)	6499(2)	14(1)
C(4)	5996(3)	4570(3)	6779(2)	14(1)
C(5)	5549(3)	5796(3)	6232(2)	13(1)
C(6)	6534(3)	6982(3)	6389(2)	17(1)
C(7)	6109(3)	3901(4)	3610(2)	28(1)
C(8)	4619(4)	5903(3)	3638(2)	31(1)
C(9)	3485(4)	3747(4)	3591(2)	36(1)
I(1)	5693(1)	9695(1)	4032(1)	18(1)

Table 3. Selected bond lengths [Å] and angles [°] for **(4.99)**

O(1)-C(1)	1.410(3)
O(1)-C(5)	1.440(3)
O(2)-C(2)	1.418(3)
O(3)-C(3)	1.422(3)
O(4)-C(4)	1.429(3)
O(5)-C(6)	1.432(4)
N(1)-C(1)	1.522(4)
N(1)-C(7)	1.491(4)
N(1)-C(8)	1.501(4)
N(1)-C(9)	1.489(4)
C(1)-O(1)-C(5)	112.7(2)
C(7)-N(1)-C(9)	110.3(2)
C(8)-N(1)-C(9)	106.6(2)
C(1)-N(1)-C(9)	109.4(2)
C(1)-N(1)-C(7)	113.0(2)
C(1)-N(1)-C(8)	108.4(2)
C(7)-N(1)-C(8)	108.8(2)
O(1)-C(1)-N(1)	104.7(2)
O(1)-C(1)-C(2)	109.3(2)
N(1)-C(1)-C(2)	115.3(2)
O(2)-C(2)-C(1)	112.1(2)
O(2)-C(2)-C(3)	106.7(2)
O(3)-C(3)-C(4)	106.7(2)
O(3)-C(3)-C(2)	110.6(2)
O(4)-C(4)-C(3)	112.3(2)
O(4)-C(4)-C(5)	108.4(2)
O(1)-C(5)-C(6)	106.2(2)
O(1)-C(5)-C(4)	108.5(2)
O(5)-C(6)-C(5)	113.3(2)

Table 4. Bond lengths [Å] and angles [°] for **(4.99)**.

O(1)-C(1)	1.410(3)
O(1)-C(5)	1.440(3)
O(2)-C(2)	1.418(3)
O(3)-C(3)	1.422(3)
O(4)-C(4)	1.429(3)
O(5)-C(6)	1.432(4)
O(2)-H(2O)	0.98(3)
O(3)-H(3O)	0.8400
O(4)-H(4O)	0.98(3)
O(5)-H(5O)	0.984(16)
N(1)-C(1)	1.522(4)
N(1)-C(7)	1.491(4)
N(1)-C(8)	1.501(4)
N(1)-C(9)	1.489(4)
C(1)-C(2)	1.533(4)
C(2)-C(3)	1.535(4)
C(3)-C(4)	1.520(4)
C(4)-C(5)	1.522(4)
C(5)-C(6)	1.515(4)
C(1)-H(1)	1.02(4)
C(2)-H(2)	0.94(4)
C(3)-H(6)	1.07(4)
C(4)-H(4)	0.99(3)
C(5)-H(5)	1.06(4)
C(6)-H(6A)	0.97(2)
C(6)-H(6B)	0.97(2)
C(7)-H(7A)	0.9800
C(7)-H(7B)	0.9800
C(7)-H(7C)	0.9800
C(8)-H(8A)	0.9800
C(8)-H(8B)	0.9800
C(8)-H(8C)	0.9800
C(9)-H(9A)	0.9800
C(9)-H(9B)	0.9800

C(9)-H(9C)	0.9800
C(1)-O(1)-C(5)	112.7(2)
C(2)-O(2)-H(2O)	108(2)
C(3)-O(3)-H(3O)	109.00
C(4)-O(4)-H(4O)	112.3(17)
C(6)-O(5)-H(5O)	117.6(19)
C(7)-N(1)-C(9)	110.3(2)
C(8)-N(1)-C(9)	106.6(2)
C(1)-N(1)-C(9)	109.4(2)
C(1)-N(1)-C(7)	113.0(2)
C(1)-N(1)-C(8)	108.4(2)
C(7)-N(1)-C(8)	108.8(2)
O(1)-C(1)-N(1)	104.7(2)
O(1)-C(1)-C(2)	109.3(2)
N(1)-C(1)-C(2)	115.3(2)
C(1)-C(2)-C(3)	108.0(2)
O(2)-C(2)-C(1)	112.1(2)
O(2)-C(2)-C(3)	106.7(2)
O(3)-C(3)-C(4)	106.7(2)
C(2)-C(3)-C(4)	113.0(2)
O(3)-C(3)-C(2)	110.6(2)
O(4)-C(4)-C(3)	112.3(2)
O(4)-C(4)-C(5)	108.4(2)
C(3)-C(4)-C(5)	109.2(2)
O(1)-C(5)-C(6)	106.2(2)
C(4)-C(5)-C(6)	112.5(2)
O(1)-C(5)-C(4)	108.5(2)
O(5)-C(6)-C(5)	113.3(2)
O(1)-C(1)-H(1)	109(2)
N(1)-C(1)-H(1)	106.6(17)
C(2)-C(1)-H(1)	112(2)
O(2)-C(2)-H(2)	106.3(19)
C(1)-C(2)-H(2)	113.5(19)
C(3)-C(2)-H(2)	110.2(19)
O(3)-C(3)-H(6)	112.2(19)
C(2)-C(3)-H(6)	106.1(16)

C(4)-C(3)-H(6)	108(2)
O(4)-C(4)-H(4)	108.6(16)
C(3)-C(4)-H(4)	108.9(17)
C(5)-C(4)-H(4)	109.3(17)
O(1)-C(5)-H(5)	105.5(16)
C(4)-C(5)-H(5)	112.5(19)
C(6)-C(5)-H(5)	111(2)
O(5)-C(6)-H(6A)	103.6(18)
O(5)-C(6)-H(6B)	109.5(17)
C(5)-C(6)-H(6A)	109.5(17)
C(5)-C(6)-H(6B)	108.6(17)
H(6A)-C(6)-H(6B)	112(2)
N(1)-C(7)-H(7A)	110.00
N(1)-C(7)-H(7B)	109.00
N(1)-C(7)-H(7C)	110.00
H(7A)-C(7)-H(7B)	109.00
H(7A)-C(7)-H(7C)	109.00
H(7B)-C(7)-H(7C)	109.00
N(1)-C(8)-H(8A)	109.00
N(1)-C(8)-H(8B)	109.00
N(1)-C(8)-H(8C)	110.00
H(8A)-C(8)-H(8B)	109.00
H(8A)-C(8)-H(8C)	109.00
H(8B)-C(8)-H(8C)	109.00
N(1)-C(9)-H(9A)	110.00
N(1)-C(9)-H(9B)	109.00
N(1)-C(9)-H(9C)	109.00
H(9A)-C(9)-H(9B)	109.00
H(9A)-C(9)-H(9C)	109.00
H(9B)-C(9)-H(9C)	109.00

Table 5. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for **(4.99)** The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2a^{*2}U^{11} + ... + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
I(1)	23(1)	15(1)	15(1)	-1(1)	-2(1)	0(1)
O(1)	21(1)	13(1)	12(1)	0(1)	1(1)	-5(1)
O(4)	26(1)	15(1)	11(1)	-2(1)	1(1)	-4(1)
N(1)	16(1)	13(1)	14(1)	2(1)	0(1)	0(1)
O(5)	35(1)	10(1)	16(1)	1(1)	1(1)	-1(1)
O(3)	21(1)	12(1)	16(1)	3(1)	1(1)	-1(1)
C(4)	16(1)	14(1)	14(1)	-2(1)	1(1)	1(1)
C(3)	16(1)	11(1)	14(1)	0(1)	2(1)	0(1)
C(5)	16(1)	11(1)	13(1)	-3(1)	-1(1)	-2(1)
O(2)	21(1)	13(1)	21(1)	-3(1)	1(1)	-5(1)
C(6)	22(1)	11(1)	18(1)	-1(1)	1(1)	-3(1)
C(1)	15(1)	14(1)	11(1)	-3(1)	2(1)	-3(1)
C(2)	12(1)	12(1)	16(1)	-2(1)	0(1)	-1(1)
C(7)	25(2)	44(2)	16(1)	2(1)	7(1)	15(2)
C(8)	59(2)	16(2)	18(1)	4(1)	-3(1)	9(2)
C(9)	38(2)	48(2)	22(2)	2(2)	-10(2)	-26(2)

Table 6. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for **(4.99)**.

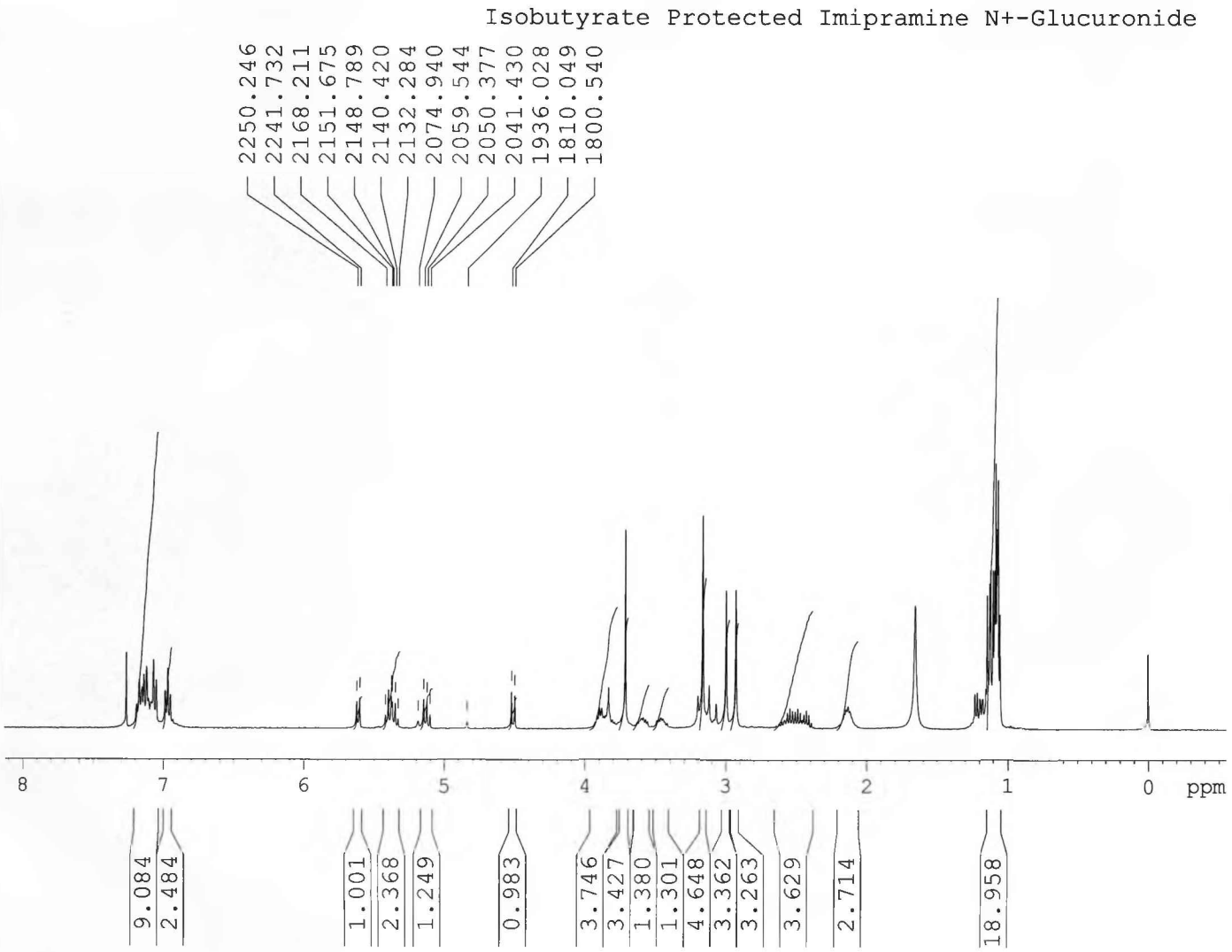
	x	y	z	U(eq)
H(1)	3660(40)	4790(40)	5170(20)	17(8)
H(2)	5940(40)	2800(30)	5270(20)	11(8)
H(2O)	4470(40)	1430(30)	4950(20)	17(10)
H(3O)	5023	1625	6948	19
H(4)	7010(30)	4360(30)	6650(18)	13(7)
H(4O)	5500(40)	4120(30)	8080(20)	17(11)
H(5)	4460(40)	6060(40)	6340(20)	18(9)
H(5O)	5750(50)	8480(30)	5538(9)	25(13)
H(6)	3980(40)	3590(40)	6690(20)	20(9)
H(6A)	6760(30)	7050(30)	7034(14)	25(7)
H(6B)	7390(20)	6870(30)	6015(18)	18(7)
H(7A)	6129	2937	3735	42
H(7B)	6929	4333	3910	42
H(7C)	6168	4051	2950	42
H(8A)	4489	5908	2974	46
H(8B)	5496	6396	3795	46
H(8C)	3790	6332	3929	46
H(9A)	3441	3876	2929	54
H(9B)	2601	4090	3870	54
H(9C)	3583	2790	3727	54

Table 7. Torsion angles [°] for **(4.99)**.

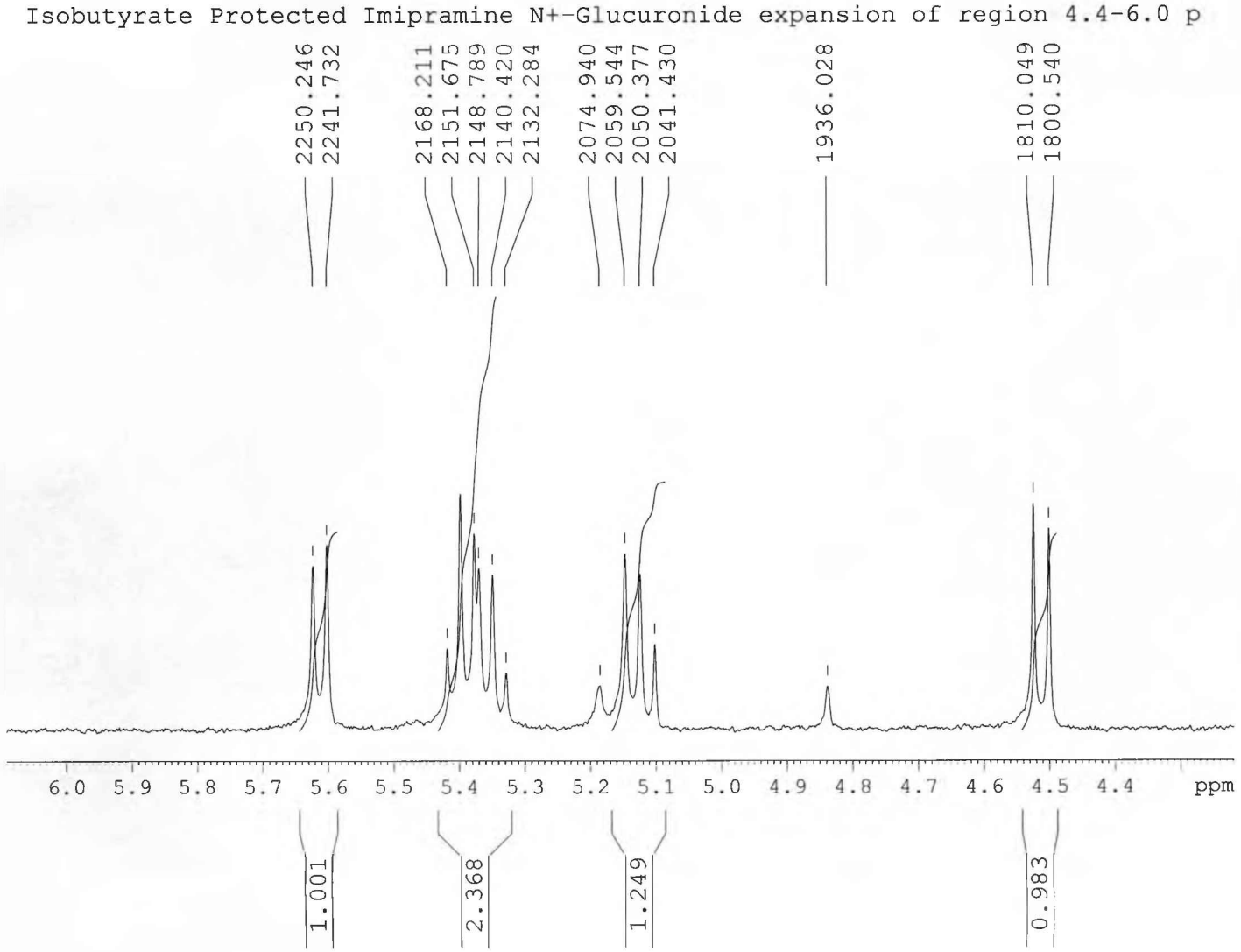
C(5)-O(1)-C(1)-N(1)	169.6(2)
C(5)-O(1)-C(1)-C(2)	-66.4(3)
C(1)-O(1)-C(5)-C(4)	65.8(3)
C(1)-O(1)-C(5)-C(6)	-173.1(2)
C(7)-N(1)-C(1)-O(1)	71.6(3)
C(7)-N(1)-C(1)-C(2)	-48.6(3)
C(8)-N(1)-C(1)-O(1)	-49.1(3)
C(8)-N(1)-C(1)-C(2)	-169.3(2)
C(9)-N(1)-C(1)-O(1)	-165.0(2)
C(9)-N(1)-C(1)-C(2)	74.8(3)
O(1)-C(1)-C(2)-O(2)	173.8(2)
O(1)-C(1)-C(2)-C(3)	56.5(3)
N(1)-C(1)-C(2)-O(2)	-68.7(3)
N(1)-C(1)-C(2)-C(3)	174.1(2)
O(2)-C(2)-C(3)-O(3)	68.2(3)
O(2)-C(2)-C(3)-C(4)	-172.3(2)
C(1)-C(2)-C(3)-O(3)	-171.2(2)
C(1)-C(2)-C(3)-C(4)	-51.7(3)
O(3)-C(3)-C(4)-O(4)	-65.8(3)
O(3)-C(3)-C(4)-C(5)	173.9(2)
C(2)-C(3)-C(4)-O(4)	172.5(2)
C(2)-C(3)-C(4)-C(5)	52.2(3)
O(4)-C(4)-C(5)-O(1)	-178.7(2)
O(4)-C(4)-C(5)-C(6)	64.1(3)
C(3)-C(4)-C(5)-O(1)	-56.1(3)
C(3)-C(4)-C(5)-C(6)	-173.2(2)
O(1)-C(5)-C(6)-O(5)	84.1(3)
C(4)-C(5)-C(6)-O(5)	-157.4(2)

6.1.4 NMR spectra

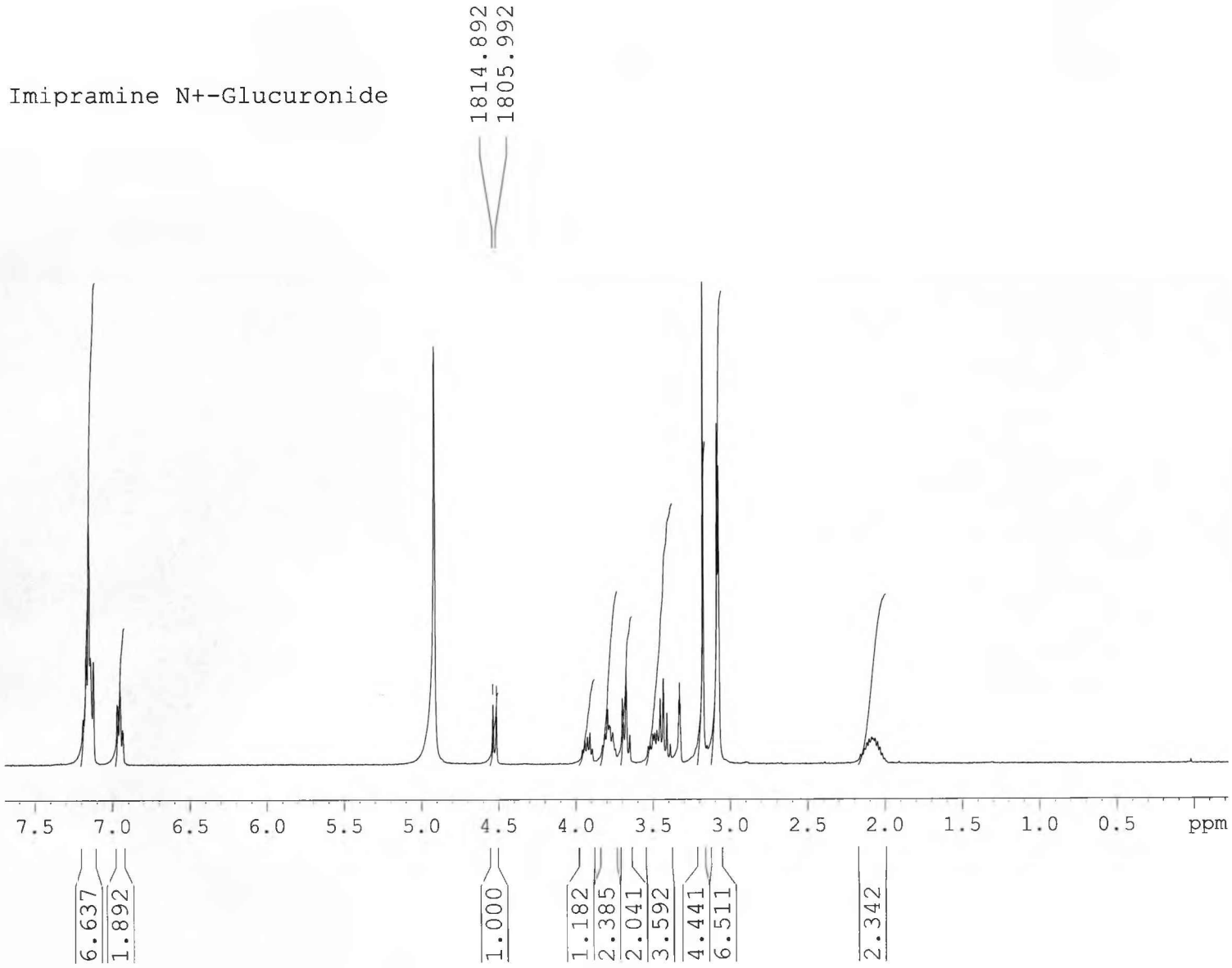
Compound (4.131)



Compound (4.131) expansion of the sugar region



Compound (4.134)



Compound (4.134) expansion